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ABSTRACT

This report by the Automation in the Medical Sciences Review Committee of the National Institute of General Medical Sciences, National Institutes of Health, assesses the current status of medical laboratory science and identifies specific areas in need of further development. (CS)

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MECHANIZATION AUTOMATION AND INCREASED EFFECTIVENESS OF THE CLINICAL LABORATORY

1976 Edition

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A STATUS REPORT BY THE
AUTOMATION IN MEDICAL LABORATORY SCIENCES-REVIEW COMMITTEE
OF THE
NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

U.S. DEPARTMENT OF
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SE 034 561

MECHANIZATION, AUTOMATION,

AND

INCREASED EFFECTIVENESS OF THE

CLINICAL LABORATORY

1976 Edition

Thomas D. Kinney, M.D.

and

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Editors

A status report by the Automation in the Medical Laboratory Sciences Review Committee of the National Institute of General Medical Sciences, National Institutes of Health.

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FOREWORD

The National Institute of General Medical Sciences supports research in the sciences basic to medicine. Research and development in medical laboratory science has been supported by the Institute in order to improve the means for studying pathophysiology as it presents itself to the clinical scientist.

In 1968, the Automation in the Medical Laboratory Sciences Review Committee of the National Institute of General Medical Sciences was established to advise and assist in the development of programs that could advance the medical laboratory sciences. More specifically, the committee has been assigned the task of reviewing contract proposals and project applications related to research and development in the medical laboratory. The committee was also charged with the specific responsibility for improving automation of the clinical laboratory. Major areas of interest included clinical chemistry, hematology, clinical microbiology, virology, and immunology.

In 1971, the committee published a status report entitled "The Mechanization, Automation, and Increased Effectiveness of the Clinical Laboratory." This report was prepared to aid the scientific community in identifying research and development that was needed to develop a completely automated clinical laboratory. Nearly ten thousand requests were honored and there were numerous inquiries regarding the possibility for an updated edition.

The present report represents a reassessment of the current status of medical laboratory science and attempts to identify specific areas in need of further development. It represents a complete revision of the original work. Some areas which appeared in the previous edition have been deleted primarily because of scientific advances, and a number of new areas have been added.

In general the report represents the combined efforts of committee members. In a number of instances, former members of the committee were called upon, either to contribute a particular section or to review the report critically. In order to acknowledge their contribution, the names of these individuals are listed along with the names of the present committee members. It is hoped that this report will be as valuable to medical laboratory scientists and industry as was its predecessor.

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THE CLINICAL LABORATORY

Mechanized clinical laboratories have become an integral part of the practice of medicine and the delivery of health care since the continuous flow analyzer was introduced in 1957. This instrument, and others which followed, demonstrated that it was possible to perform many laboratory tests by machine that hitherto had been performed manually. The acceptance by laboratory scientists and the medical profession of the fact that reliable laboratory data could be produced rapidly by mechanized instruments was almost immediate. Subsequent instrumental developments have resulted in both improved precision and greater workload capacity. Indeed, these innovations have been so successful that there is an increasing demand that all laboratory procedures be mechanized. It has not been possible, however, to design and develop new equipment rapidly enough to meet expectations.

Although past achievements have been remarkable, complete automation of any division of the clinical laboratory has not yet been accomplished despite claims of Industry. In operational terms, a completely automated laboratory would be "one which establishes positive patient-sample identification as soon as sample material is obtained from a patient and maintains this identification throughout a mechanized analytical chain and final presentation and/or recording of valid results."[1] Such a goal is some distance in the future. Nevertheless, it is common to encounter systems which mechanize two or more steps of a procedure but still require some manual intervention, such as preparing the specimen for insertion into a machine or tabulating test results from a strip chart or digital display onto a report form. At present, there remain a number of problems in

technique, instrumentation, or data processing that seriously retard the development of automated systems in almost every area of clinical laboratory practice.

This report was prepared by the Medical Laboratory Sciences Review Committee of the National Institute of General Medical Sciences. Its purpose is to aid the scientific community and biomedical industries in identifying research and development projects needed in order to further develop automation for the clinical laboratory. The report comments upon the significance of new developments in the field and points toward specific needs and neglected areas which require further development.

While emphasizing the need for further advances in instrumentation, the committee recognizes that progress in the medical laboratory sciences is equally dependent upon development of improved laboratory methods which have greater specificity for diagnosis and management of individual disease states. For many illnesses, indirect tests are performed because we simply have not discovered what to measure. No amount of refinement of existing tests will solve this problem. Given the large volume of research and development now in progress, it can be expected that an ever increasing number of sensitive and highly specific diagnostic tests will be developed.

Instrument Requirements and Related Considerations

Although it is not now possible to devise guidelines for automation of an analytical instrument that would be applicable to every situation, it is feasible to indicate certain basic characteristics of such an instrument for clinical laboratory use. It should produce accurate, precise, and reproducible results; have a capacity to perform several tests in sequence with a minimal delay time between sample entry and result: use small samples

(25 microliters or less) per analysis; provide if possible, for the entry of emergency specimens into the system without interruption of ongoing analytic procedures; and still insure positive sample identification from input to output.

It is not important whether the analytical method is based on a chemical or a physical principle so long as each step of a procedure can be identified sufficiently to allow simulation for programming and checking purposes. Too often, the approach to instrumentation for the clinical laboratory has been restricted to the mechanization of existing manual methods. Since some manual biochemical analyses are difficult to adapt for automation, more imaginative uses of procedures developed for research in physical chemistry might provide bases for improved clinical laboratory techniques. Such analyses often involve the use of specialized approaches which lend themselves readily to instrumentation and automation.

In a more immediate sense, means must be found for ensuring that relevant existing technology does, in fact, reach the clinical laboratory. This is especially true of mechanisms for error detection and methods for standardization. If possible, every step in an automated procedure should be monitored to detect any abnormal function. Alarm mechanisms should alert operators to machine malfunction, loss of specimens, specimens of insufficient quantity, and the printing of obviously erroneous results. Machines should function with minimum noise and drift; feedback control should continually compensate for unavoidable drift. Reliability and mimimal maintenance requirements should be designed into an instrument from the outset. Wherever possible modular components should be standardized so that the operator can check and replace faulty parts with a minimum of

downtime.

Ordinarily, two considerations are important in assessing the need for development of a new laboratory instrument: (1) an estimate of the importance and utility of each analysis, i.e., is this analysis(es) worth an instrumentation or automation effort?, and (2) the importance of improving an analysis which is demonstrably useful but is not well performed by existing laboratory methods. These judgments and validating documentations cannot be made by instrument companies or even non-clinical laboratory scientists in vacuo, i.e., outside the experience of the clinical laboratory and its medical environment. On the other hand, it is unreasonable to expect that scientists preoccupied with the primary responsibility of a routine service load could provide the necessary effort and innovative design and engineering expertise for developing sophisticated new automated technology. The clinical services, the laboratory, and the basic and engineering sciences all must play a role in the development of an improved clinical laboratory analytical capability. Means must be found for correlating and coordinating the clinical laboratory activities with all of the necessary adjunct disciplines which can contribute in the development of specific research programs oriented towards solving specific laboratory problems. This research effort must be protected from the service load responsibility. Explicit provision must be made for research to develop and prosper within the context of the clinical laboratory.

Intelligent cooperation with capable instrument manufacturers is important for the transition from pilot models to production instruments. The suitability of an instrument can be determined only by bench evaluation of its performance. It is worth noting that major innovations invariably

trace back to a single individual whose care and nurture assured completion of the project.

Another primary requirement for any instrumentation development is the parallel development of suitable reference standards. Such standards serve for calibration and control of the analysis and allow intra- and inter-laboratory comparison of results. The requirements for the use of such calibration standards should be mandatory for both the instrument manufacturer and the user.

One pressing need in clinical laboratories is sufficient factual information about the accuracy, precision, reliability, and utility of laboratory instruments, including directions for their calibration. Many new instruments designed for the clinical laboratories have been inadequately field-tested prior to active marketing by the manufacturer. Consequently, time and money are often wasted in the busy hospital laboratory in putting such instruments into operation--either because the instrument is found to be in need of extensive adjustment or modification or because it is found to be incapable of performing the task for which it was purchased. With improved field evaluations, prototype laboratory instruments could be redesigned and modified before production models are released to the general market. There is no doubt that more careful studies of prototypes would eventually improve accuracy of laboratory determinations and decrease frustration of laboratory personnel.

More attention should be given to safety precautions. At the present time, serious electrical shocks among laboratory personnel using faulty equipment are not infrequent. To eliminate such hazards, new equipment should be validated for safety in terms of units as well as in total packages.

Since other hospital and laboratory accidents occur because instruments from more than one manufacturer are coupled together without proper grounding procedures, certification of equipment should be required and should be validated by a non-partisan laboratory. Electronic manufacturers currently maintain underwriter laboratories but there is at present no similar mechanism for certification for electrical safety in the medical field.

Development Objectives

In order to provide the quality and quantity of laboratory services needed for the best patient care, three basic objectives must be recognized:

(1) delivery of uniformly high quality laboratory service at all times,

(2) expansion of mechanized processing to provide higher quality service at an acceptable cost, and

(3) improved correlation of laboratory and clinical information for optimal use.

A rational approach to the development of automated clinical laboratories should include the following goals:

A. Upgrade existing systems and approaches

As is the case in any dynamic clinical laboratory, responsible staff constantly strives to achieve this never-ending goal.

This objective is dictated by a continuing workload increase and should, in many instances, be approached as an interim solution because many laboratory tests and instruments now in use ultimately should be replaced. Until replacement tests can be devised and validated, laboratories must "make do" with whatever improvements are now possible. Some of these improvements involve mechanization or automation of test analyses, incorporation of appropriate patient-sample identification, and instrument

miniaturization, and a more extensive use of computers. Included in this category would be instruments for semi-automated methods requiring some manual sample preparation that are suitable for use in smaller hospitals and for intermittent or emergency use. The criteria for judging the suitability of these instruments should be immediate availability, rapid completion time, ease of calibration and operation, and high reliability.

It should be recognized that there are apt to be certain pitfalls in any decision to upgrade an existing method. Among these may be a tendency to tie-in traditional instrumentation, for example, colorimetry and spectrophotometry, which still dominate analytical procedures, rather than to consider fluorometry or isotopic techniques which have now supplanted many older procedures. There is also more risk of developing mechanized equipment for tests which are less specific in diagnostic usefulness than might otherwise be available.

One of the more successful attempts to upgrade existing systems is the direct continuous monitoring of reaction rates to measure the activities of enzymes in serum. Major manufacturers have adopted this approach with significant improvement in the quality of test results. The field is still severely limited by the absence of standardized enzyme preparations and until these are available in some definable form of purity, results will continue to be based on experimental observations from a number of laboratories-- never entirely definitive.

Recent activities of the various international professional societies are pertinent in this regard. Thus, the International Federation of Clinical Chemistry has developed some general guidelines and philosophies concerning the standardization of measurements of enzyme activities in human serum and

plasma. By applying these "standards of methodology," improved comparability of analyses both within each laboratory and between laboratories could be achieved.

B. Develop new methods, procedures, instrumentation, and systems to assist the clinical laboratory in attaining excellence, high productivity, and cost effectiveness

The available reservoir of scientific knowledge and technology is often not applied to the solution of clinical laboratory problems. The establishment of a "proof of principle," for a new instrument is relatively inexpensive as compared to the effort and financial support required for reducing to practice a specific design of the instrument. In each instance reliable criteria for selecting the best approaches, and projects with the most promise of substantial improvements in laboratory performance must be developed.

Laboratory automation in the past involved physical as well as chemical measurements not only of blood serum but of red cells and individual varieties of white cells. Further effort is needed to devise instruments capable of performing urine analysis in greater detail than any available to date; and chemical analysis of tissues obtained by biopsy techniques. In the area of analysis for individual molecular species new and refined types of separation techniques may be possible by molecular separation and identification by ion exchange chromatography, gel filtration, and gas and/or high pressure liquid chromatography. A variety of instruments, not generally used by clinical laboratories, have been developed and refined employing physical measurements. These have been used to study biological material and systems for research purposes and appear, in principle, to be

amenable to automation. Examples of these are low temperature and rapid scan ultra violet and visible spectrophotometry, nuclear magnetic resonance and electron paramagnetic resonance, and optical rotatory dispersion and circular dichroism spectroscopy.

Calorimetry is an example of a potentially useful physical measurement that can be applied to the quantitation of biological reactions. This technique is based on the general principle that all natural chemical and physical changes are accompanied by a thermal effect. Although this thermal effect is often exceedingly small, it does represent an enthalpy or energy change for a process and its measurements can contribute to our understanding of the process involved and for analytical purposes. At the present time, calorimetry is not used in the clinical laboratory because the instruments available, until recently, have not been sufficiently sensitive or discriminatory for many biological measurements. Microcalorimeters[2] have now been developed that are capable of detecting the minute thermal changes produced when an isolated enzyme contained in one to ten microliters of serum is allowed to react with an added specific substrate. Thus, at least on a limited basis, microcalorimetry shows promise as a clinically useful laboratory tool.

C. Identify areas of high promise in basic research and development

While many marked improvements can and must be obtained through the direct application of present knowledge, it is evident that the new and revolutionary developments of the future will be based on knowledge we now lack. The broad outline of these advances can sometimes be envisioned clearly enough to identify the nature of the basic research that must be done to achieve them. Progress may be markedly accelerated by judicious

and thoughtful choice and adequate funding of innovative projects. As in all basic research, the research mode in the clinical lab should be free of the constraint of immediate therapeutic relevance but must never lose sight of it.

D. Training of laboratory personnel

Maximum utilization of the clinical laboratory depends on adequate staffing by well trained professional and technical personnel. The expanding variety of scientific knowledge applicable to clinical laboratory problems makes it unlikely that traditional academic procedures as now constituted will provide the kind of training that is needed. For example, the automation of clinical laboratories is placing new demands on laboratory directors.[3] These professionals now need an intimate and detailed knowledge of the special characteristics of automation techniques at both the theoretical and practical levels. A new kind of scientist who is devoted to the management and improvement of the clinical laboratory will be required for the future. Otherwise, it will not be possible to provide the leadership that the clinical laboratories must have if they are to rise above mere technical competence. Scientists trained in this manner should understand normal and abnormal human biology at a level which hopefully may enable them to make contributions to the understanding of disease and to the development and use of laboratory approaches to essential measurements.

While it has been repeatedly observed that the introduction of automated devices into a clinical laboratory increases the numbers of tests performed without reducing the levels of skills required of laboratory personnel, experience has demonstrated that the use of an automated instrument is no guarantee in itself as to accuracy of data. Because the detecting systems

incorporated into such instruments are usually based on the same chemical and physical principles as those used in the conventional non-automated test, the instrument requires supervision by a soundly trained and careful operator, otherwise the automated instrument may drift out of control and produce inaccurate data. In most instances, laboratory personnel must be trained in considerable depth to operate the automated equipment efficiently and with sufficient understanding to insure reliable data. Only in a few instances, where the rate of test output is low and operation is simple, is it possible to employ technical help with less training. Nevertheless, the fact remains that the more complex and costly automated equipment requires a superior trained operator for successful operation. Further, it is often necessary to employ additional personnel with skills heretofore foreign to the laboratory, such as key punching and computer programming.

PROBLEMS NEEDING SOLUTIONS

In general, there are two types of problems that must be solved if we are to proceed in an orderly fashion toward the goal of effective and efficient automation. Of first importance are the problems that are common to all laboratories. These include resolution of the question of reference values for the substances to be tested; the establishment of standard reference materials and reference methods; the development of fail-safe methods of quality control for monitoring the accuracy of laboratory tests; positive patient-sample identification; error-free reporting of data; miniaturization of equipment; and demonstration of cost effectiveness.

The second group of problems involves tests or techniques that relate specifically to laboratory and clinical disciplines as clinical chemistry, hematology, microbiology, etc. New developments in these disciplines include tests developed on the basis of new knowledge obtained from laboratory research or developed specifically for the solution of problems relating to clinical diagnosis and therapy.

In this section, discussion will be concerned with unresolved problems that require universally applicable solutions so that uniformly comparable data can be obtained from all laboratories.

Reference Values

A discussion of automation would not be complete without the consideration of reference values, (formerly known as "normal" values) because, by their very nature, automated devices should "flag" or identify specimens that yield unexpected clinical data; that is, data found to be outside of the limits normally found for comparable individuals under similar conditions.[4,5,6]

The great increase in the use of mechanization in clinical laboratories during the recent past has emphasized the need for definitions of reference values for laboratory tests. At present, there are no universally acceptable reference values transferable from one laboratory to another and from one age group or sex to another. The data for a given patient obtained from two different laboratories many times seems to be inconsistent, thus making it difficult for the physician to interpret and use the information. To resolve this dilemma the physician must order repeat laboratory work from a laboratory that uses reference values with which he is familiar--a process that is both expensive to the patient and requires a duplication of effort.

Equally important, a definition of reference values is needed in order to validate the results of "screening" healthy populations for latent disease. The commonly accepted reference range in use today is obtained by purely statistical methods based on two Gaussian distributions of each parameter measured: one for well individuals, the other for sick patients, with the assumption that these two curves can be easily separated mathematically. Elvebach et al.,[7] point out that there is no mathematical, statistical, or other theorem that enables us to predict the shape of the distribution of physiologic measurements, moreover the distributions of results in healthy persons cannot be described adequately by a Gaussian distribution. If the other known variables, such as age, race, sex, diet, geographical location, genetic variations, diurnal and seasonal changes are superimposed on the already artificial distribution curve, it becomes impractical to establish reference values for every parameter applicable to every individual under all conditions.

The Expert Panel on Relative Values of the International Federation of Clinical Chemistry (IFCC) recommends strongly that the use of the term normal be avoided with reference to values of tests performed on healthy people. The reference value of a test result may be quite different for a bed patient in a hospital who is not ill but is awaiting minor surgery or confined for psychiatric reasons from that of an ambulatory worker who is in vigorous health. There is a need for many sets of relative values relating to age, sex and other demographic information. These should be well documented because in health management and medical care the physician needs different sets of values for different sets of conditions. Some of the categories for which such sets of relative values should be established are groups of people in exceptionally vigorous health, general unsick populations (a group from which traditional reference ranges are obtained), persons of different ages, particularly children and the aged, sick patients and confined individuals, and particularly values for patients with specific diseases. With present day knowledge of health and disease, it is futile to continue the practice of categorizing laboratory test results into two groups, normal and abnormal. The IFCC panel is preparing to publish recommendations regarding the classes of relative reference values in the near future. These should establish the requirements for documentation and use of each class of relative values.

A number of laboratories in different regions of the country have collaborated in a study of clinical laboratory test profiles on healthy men and women. Results show that when carefully performed, blood levels of the constituents measured change so little in healthy individuals that small but perhaps significant changes which do occur may be obscured by the larger variations due to analytical error inherent in many of the laboratory techniques

now in use.[8] Until these variables can be controlled and the analytical error reduced to at least the point where physiological variaitons in well individuals can be observed, any attempt to establish reference values for automated instruments will be unrewarding.

It may be that reference values will be defined in terms of the individual patient himself, and that relative changes in any blood parameters will be considered significant only when compared with previous values attained on the same person.

Standard Reference Materials

Basic to the successful exploitation of analysis and measurement is the standard reference material used for calibration. The "standard" has long been recognized as the indispensable factor in the validation of all analytical procedures. Standards used in clinical laboratories are either primary, secondary, or in some cases, arbitrary standards established by mutual consent on the part of the members of the disciplines. A survey conducted in 1968 by a subcommittee of the Committee on Analytical Chemistry, Division of Chemistry and Technology, National Academy of Sciences of the National Research Council found a great need for standard reference materials in clinical chemistry laboratories. Subsequently, the College of American Pathologists and the American Association of Clinical Chemists asked the National Bureau of Standards (NBS) to develop standard reference materials (SRMs) certified as to purity for the clinical chemistry laboratories. These reference materials now make it possible for medical laboratories to calibrate instruments, test procedures, and relate their analytical results to a common base. Laboratories which routinely use SRMs can also claim significant progress in their efforts to achieve the high standards of accuracy and

precision demanded by modern medicine. An important by-product of the widespread use of standard reference materials hopefully will be more valid interlaboratory comparisons than hitherto obtainable.

Further pressure for use of SRMs in all clinical laboratories was created in 1967 by passage of the Partnership in Health Act enpowering the Center for Disease Control in Atlanta, Georgia, to evaluate laboratory performance and to close laboratories if they failed to measure up to predetermined standards.

The first two basic SRMs produced by the NBS for the clinical laboratory were cholesterol and creatinine. These were followed a year later by several other standards as funding was provided for their purification and production through an interagency agreement with the National Institute of General Medical Sciences of the National Institutes of Health. During the past five years, 18 others, including urea, uric acid, calcium, bilirubin, d-glucose, potassium, sodium, cortisol, lithium, and several of the important biological buffers have been produced.[8] These materials are now the point of departure of the proficiency testing program run by the Center for Disease Control. As an example of what can be done using the SRMs as points of departure: in 15 hospitals, the routine use of a standard reference material (calcium) and a specified reference method for that determination has reduced the error in the serum calcium determination between the hospitals to less than two percent.[9]

The professional societies concerned with clinical chemistry analyses are now actively formulating requirements for additional standards and are looking to the NBS for their development. To achieve these requirements, many of which are directed into fields beyond those for which the NBS has

historically received budgetary support from the Department of Commerce, will necessitate an extension of the Bureau's efforts and resources. An effective program for the development of standard reference materials for proteins, coenzyme, and enzyme analyses will require a strong competence in protein biochemistry and enzymology, and would constitute an extension of NBS work already in progress towards SRMs for gas analysis, pH, ion specific electrodes, spectrophotometry, activation analysis, and the development of high-purity reagents.

Mechanization of laboratory procedures has reinforced the importance of standard reference materials for the achievement of greater precision and accuracy. There is no question that clinical chemical methods of the future must rely heavily on certified standard reference materials to obtain the necessary reliability for physiological interpretations and for the comparison of patient data obtained at different times by the same or different laboratories.

Standard Reference Methods

Many of the arguments for standard reference materials can be used with equal justification for standard reference methods. Reference methods for clinical laboratory tests are necessary in order to compare analytical results obtained by different methods and for the evaluation of new techniques. Every laboratory need not be required to use the same method but all methods used must provide results within the degree of accuracy defined by the reference methods. Indeed, it would be inadvisable to expect all laboratories to use exactly the same procedures for performing tests for two reasons:

(1) The instrumentation required for a method used in a large facility performing several hundred analyses daily may employ instrument

reagents which are unsuitable or too expensive for the smaller laboratory which performs only ten analyses per day.

(2) Strict adherence to uniform regulations would prevent the development of new methodologies and would be resisted by both scientists and clinical laboratory personnel.

The sine qua non for a reference method is proven and demonstrated accuracy. Absolute accuracy, implying the use of methods with no systematic biases, is an unattainable goal. It is importance to realize that a rise in cost is an exponential result of increasing accuracy. Only that degree of accuracy required for the end purpose should be sought, making allowance for further advances in the current state-of-the-art. A good rule of thumb is to strive for a reference method with precision and accuracy that is three times better than the physician requires.[9]

The reference method should be used periodically to check the accuracy of the routine method which is often used to turn out a large volume of results. Of course, if the reference method can be used in the laboratory and meet the requirements for speed, ease of operation, and low cost as well as the routine method does; then the reference method should be the method of choice.

Drug Influence on Laboratory Tests

The increasing use of multiple therapeutic drugs for the same patient has presented considerable problems for clinicians in the interpretation of data. Not only may drugs interact chemically so that their physiological effects are reduced or augmented, they may also produce sufficiently serious interferences in laboratory procedures to cause incorrect estimates of the concentration of certain constituents in biological fluids. Furthermore, the

physiological (and real) effect may not be in the same direction as the laboratory findings. Even aspirin, vitamins, and barbiturates may have marked effects on laboratory procedures. Indeed, the effect of vitamin C in the doses now advocated by some physicians can be enormous on analytical procedures that depend on measurement of the reducing properties of a compound.

To facilitate interpretation of drug influences on laboratory tests, a computer based system has been developed to correlate automatically the drugs administered and possible effects on laboratory tests.[10] The central file used for this is available in printed form for physicians and laboratory scientists. To date this file reflects the influences of over 17,500 drugs on clinical chemistry tests.

Positive Patient-Sample Identification System

It is clear that the development of effective methods for positive patient-sample identification is of top priority. There is a great need for dependable automated systems that link each specimen to the patient from whom it has been obtained and subsequently to the tests performed on the specimens and finally to the laboratory results. In addition to human-readable sample identification, the linkage should include machine readability for effective computer storage and retrieval. Accuracy, reliability, low cost, flexibility, convenience, and speed are the obvious prerequisites of such a system.

The ultimate sample identification system, in order to function effectively, must take all parts of the hospital system into account. The form and format in which the patient's sample is presented for analysis is not just a matter of concern for the laboratory and the patient; it directly involves the physician, the nursing staff, the medical records service, and the business office.

The system begins with the assignment of a unique patient number on admission. The clinical laboratory sequence begins with the writing of an order by a physician, and it is not completed until the information requested from the laboratory is returned to the patient's chart and the business office has the necessary record for billing and cost accounting. The number of intervening steps in this complicated process may be impressive. At one center, where the matter was studied, it was found that 28 steps were involved in this process. The time delays are often appreciable and can be critical in various areas such as accident rooms, intensive care, and coronary care units.

Positive, foolproof, automated methods of controlling the flow of information associated with individual samples are needed to replace error-prone manual and semi-automated techniques. There are a number of commercial automated systems now in experimental states of development. One such system[11] which offers promise of great flexibility and reliability involves the use of the bar code commonly associated with chain grocery stores. Until such instruments are commercially available, however, it is likely that progress will be restricted to the mechanization of the information processing aspects of existent and emerging instrument systems.

Data Processing and Reporting

(a) Input devices for sample identification data at present include typewriters, mark-sense and punched card readers, and specially designed keyboard consoles for specific purposes. For example, special keyboards coded for cell types, either for differential cell identification and enumeration for peripheral blood, or for bone marrow examinations, are in daily use and are available commercially.

(b) Data and information output are accomplished by a variety of devices, such as typewriters and high speed printers. Typewriters are slow and quickly produce a bottleneck in even the smallest laboratories. High speed printers are expensive but efficient and permit more rapid production of reports, work lists, and quality control data. Cathode ray tube displays of data are used within some laboratories and in a few outpatient clinics to provide ready access to laboratory information.

(c) Data processing programs vary from elementary sorting and listing of final results; as transmitted to the computer by input devices (cards, tapes, and typewriters) to more sophisticated programs that receive and correlate the patient sample with the correct identification data and raw test results. Some of the more sophisticated programs provide for appropriate computations on the raw data and for a comparison with similar data which has been previously obtained on the same patient. These programs also permit comparison with standards to assure quality and correlation and provide a cumulative summary of all test results for a given patient in a designated period.

Computer programs which can provide more effective use of laboratory data are needed. This would appear to be a fruitful field for exploration. For example, general and widely useful computer programs are needed for biological or physiological validation of specific patient data, for correlating groups of related data, for integrating new clinical information, and for management pointers oriented to the specific patient. A sharp distinction should be made between data manipulation and data reduction to information. The terms are self explanatory but it should be pointed out that most efforts to date have been in the field of data manipulation.

Physicians are now expected to remember reference ranges for hundreds of tests. Unfortunately, so-called reference ranges are modified as analytical procedures change, as new units of measurements are introduced, or when additional information permits "new" reference ranges to be identified. For example, when unusual changes which correlate with age, sex, weight or other demographic characteristics are discovered. The seriousness of this problem is illustrated by the fact that the units in which some serum enzyme activities are expressed have changed three times during the last few years. For example, glutamic-oxaloacetic transaminase activity in serum was initially expressed as Karmen units which were linked to a change in the absorbance of the reaction mixture with time. This mode of expression was changed to International Units reflecting the micromoles of substrate consumed per minute, and finally as Standard International Units in which enzyme activities will be expressed as the number of nanomoles of product formed or substrate consumed per second. It would appear that within this decade the use of the International System of Units (SI) will become widespread in the clinical chemistry laboratory.[12] The increasing acceptance of the SI by many countries means that SI units are already in daily use for reporting laboratory data and are used exclusively in several foreign publications. In general, the SI provides a more unform system for reporting clinical laboratory data than traditional means. For example, molar concentrations of enzymes or substrates would be used in place of mg%, mg/100 ml or mg/dl, that is, 5.6mmol/l instead of 100mg% blood glucose. The SI system utilizes the unit gram or submultiples of gram per liter (g/l). This presupposes that mass concentration is measured. The unit mole(mol) and its subunits presupposes the amount of a substance: mol/l presupposes substance

concentration and the units unmol.s $^{-1}$/l and nmol.s^{-1}/l (used to report enzyme activity) presupposes catalytic concentration.[12]

A uniform reporting system with a well thought out format is essential if physicians generally are to make full use of laboratory data. This means that the outputs from a variety of instruments, computers, and software cannot be used to their maximum advantage until the clinical laboratory scientist and the practicing physician agree on units and formats.

While much effort has been spent on the development of data manipulation systems, a complete, widely accepted, and entirely satisfactory system is yet to be developed. The industrially desirable objective of a turnkey laboratory operation in which all instruments, specimen containers, and reagents are provided by one manufacturer should be discouraged. The above items should not be interfaced in such a proprietary manner that other sources of supplies and equipment are excluded.

Systems Approach

As the majority of human ills come to be better understood in terms of their biochemistry and physiology, batteries of sophisticated tests or other discriminating methods will be demanded to identify each one. Many minor genetic diseases or predispositions are already known which will give rise to genetic typing of considerable complexity. Even a specialist will not be aware of the reference values for all tests or the implications of all deviations, especially when one deals with clusters of interrelated factors. Experience has shown that no one individual can draw all the possible conclusions from the mass of data, evaluate each conclusion in terms of probabilities, and indicate what additional tests should be done. The full potential of data which could be obtained cannot be realized with current

data handling methods. Complete mastery of very complex computer-interfaced analytical systems will ultimately be required to fully utilize the medical knowledge and expertise available. If the clinical experience and knowledge of the most competent physicians could be collected and be applied, possibly by use of a computer, in the assessment of a patient's total clinical and laboratory findings, the information obtained would be more valuable than that obtained by interpreting singly the results of a complex array of many tests. For instance, many physicians now consider a blood sugar level as diagnostic without regard for other important clinical parameters which, taken together, could provide a better basis for diagnosis. The time is rapidly approaching when there will be a complex array of parameter that must be coordinated in order to arrive at a more accurate diagnosis. This task can only be accomplished by the computer. Questions that need to be answered by the automated clinical laboratory are not ones such as "What is the concentration of a certain substance?" but rather "What is the disease process--when does it first manifest itself by values outside of the normal range, and which parameter changes first?" Ultimately the information obtained by the computer, from analysis of the complex array may result in a printout stating the probabilities for several diseases. The physician can then apply his experience and judgement to decide which probability is most applicable to the patient under consideration. The final diagnosis will still be based on his knowledge but he will have a much larger informational basis for making an intelligent decision and the patient will benefit. This is somewhat analogous to the way in which missiles are checked out step-by-step and system-by-system. Years ago, with torpedos, experts personally checked out each of the many different parts. At present, a

Polaris Missile is checked by a computerized system that examines a number of functions simultaneously. If the missile is not functioning properly, the computer will ask for more data, check more parts, and determine the source of the error. The experience of hundreds of engineers has been reduced to a program in a technical device which allows very rapid decisions, thus making it possible to determine among thousands of parts exactly which one is not functioning properly. Theoretically, it should be possible to use an analogous approach to detect a malfunctioning component in the human body.

Thus when equipment becomes available that can automatically perform large numbers of different tests, it will be possible to program computers to react to a list of the patient's symptoms and indicate specific tests that should be performed. On the basis of these initial test results, the computer can then indicate whether a second set of tests should be performed, and what they should be. The final step will be to provide the physician with a list of probable and possible diagnoses based on the laboratory data correlated with the symptoms.

A practical problem confronting the automated clinical laboratory diagnostic unit is the "flagging" of abnormal results. Automation implies the use of a computer to scan all of the results on any given patient and identify those that are abnormal. Within the conventional range of reference values which is obtained by averaging all non-sick individuals, an incorrect reference value is not unusual. No ready solution to this problem is available to date. Convention dictates that we continue to compare test values against the non-sick average value for the patient.

Miniaturization

Technological advances in computers and sensors, in electrical components such as motors, switches, controls, data displays, and in materials and

design should make possible the construction of instruments that are considerably smaller and yet more flexible in design than those presently in use. A number of the advantages that may result from miniaturization are:

Microsampling - Even today the large number of blood chemical analyses which are available require judicious selection to avoid collection of excessive amounts of blood. Miniaturization of instruments usually permits the assay to be performed on much smaller samples and, this, in turn, permits the analyses of a larger variety of individual tests on each patient sample.

Reagent requirements - Reagents in the clinical laboratory constitute a significant fraction of the assay costs. By reducing sample and reagent volumes by a factor of ten, it is evident that significant savings can be effected. This is especially true for the many new enzyme radioimmunological and fluorescence antibody assays for which the reagents are very costly.

Ease of standardization - Miniaturization has always resulted in an increase in the number of components integrated into one replaceable part. Operational amplifiers, integrated circuits, and printed circuit boards are examples. As larger circuits and systems are miniaturized, an instrument finally becomes a manageable collection of functional modules. If properly designed, each could be checked by a relatively inexperienced operator and replaced if found defective. An excellent example of this trend is found in Nuclear Instrumentation Modular Systems (NIMS) developed jointly by the Atomic Energy Commission and the National Bureau of Standards. A complete counter can be assembled from preamplifiers, amplifiers, scalers, and related components from different

manufacturers. Although electronics will likely become more complex, reliability of components is now at a point where laboratory instruments are dependable and should last a long time. Insistence by users on standardization of components according to continually revised criteria can offset any possible tendency of manufacturers to build in obsolescence because reliability and performance of components can be compared by the user.

Space saving - Usually more than 50 percent of bench space in the clinical laboratory is taken up with various types of instruments. Any type of construction is costly today and the price of building laboratories is especially high. Obviously, anything that can save laboratory space will result in significant economy.

Easier maintenance - Repairs can be minimized and simplified by combining miniaturization with a decrease in the number of replaceable parts. Amino acid analyzers, for example, are presently repaired in the field, a costly and time consuming process. Industry has long recognized that it is, in general, more efficient to move a small instrument to its repair specialists in a centralized location rather than to pay a repairman to come to a laboratory to repair it. When problems arise, a machine in operating condition is dispatched at once to the user, who returns the defective one in the same container. Such arrangements clearly reduce the cost of operating the laboratory and increase its efficiency.

Lower cost --Standardized miniaturized systems should ultimately be less expensive than present large systems because of ease and low cost of maintenance.

Sample Accuracy

Emphasis will increasingly be on the analysis of samples having a small fluid volume (1-20 µl range). Special consideration must be given to the forces which govern manipulation of such small volumes, e.g., which determine the wetting of surfaces, an important factor in transfer, mixing, and measurement. In some respects this is a new field and its importance should be more widely understood.

Absorbance measurements with such small samples require special optical systems, methods for eliminating bubbles and stray particles, and a means for cleaning small cuvettes if they are to be reused. If electrodes are to be used for measurement, drift, calibration, and specificity are problems. If enzymes are used as reagents or as essential components of electrodes, problems of enzyme stability or of enzyme "poisoning" require careful consideration.

Obviously, much additional work needs to be done on the chemistry of many analytical reactions. For example, emphasis should be given to carefully designed kinetic analyses which help to lessen or eliminate many problems associated with blanks and interfering substances.

Immediacy - The most efficient part of the diagnostic process is that which occurs in real time, i.e., during the time the physician is actually with the patient. As questions and possibilities occur to the physician, he may look for answers by physical examination, observation, or questioning. He is frequently left with possibilities requiring X-ray studies or other special laboratory tests, and he delays further decisions until the results of such work is available to him. The results still may not be definitive and more studies entailing further

delays may be required. It is self evident that if the results of the various tests and studies could be obtained during the initial diagnostic work-up while questions are being asked and possible diagnoses are being considered, much time and expense could be saved. One of the distant possibilities is real-time clinical chemistry in which the physician or his trained assistant obtains the needed results within minutes using miniature automatic diagnostic machines. These would be somewhat analogous to the cameras developed by Land, where the awesome complexity entailed in the manufacturing process allows the final steps taken by the user to be simple, reliable, and rapid. These considerations more than others justify contained emphasis on miniaturization and speed.

Cost Effectiveness

Documentation of well defined cost benefits is scant and applies only to partial systems or to individually automated tests. Information available from informal discussions with laboratory directors, however, indicates that in large laboratory operations, automation and data processing are effective in reducing operational costs.

Several careful and detailed studies of the cost benefits and service effectiveness of automation in small and medium sized laboratories are needed as guidelines for future development. It must be recognized moreover that service effectiveness is possibly more important than cost effectiveness. For instance, if automation and data processing improve the accuracy and reproducibility of 25 percent of all tests performed and at the same time reduce errors in results from eight or ten percent down to one or two percent, the improvement in quality of service and benefit to patients is difficult

to calculate in terms of cost dollars.

Although specific documentation is not yet available, the experience of knowledgeable laboratory directors in handling increasing workloads without increasing technical personnel indicates that in larger institutions with annual laboratory budgets approaching $1 million or more, effective automation saves $150,000 to $200,000 per year.

In smaller laboratories operating at levels of less than $500,000 per year total budget, cost effectiveness of computerized automation at current prices is questionable. Useful systems costing $20,000-$75,000 per year in leased equipment and computer time, however, would be financially tolerable and could increase productivity and efficiency.

At this time, although expensive automation for the smallest laboratories is not cost effective it is service effective and judicious usage is appropriate. There is still need to develop mechanical devices to save the labor and the time of the few technicians involved. There is also a need to provide low-cost, leased terminals for computational and reporting services in these laboratories. Theoretically the time saved would lower the costs.

PROBLEMS INVOLVING SPECIFIC LABORATORIES

This section will delineate some of the unresolved problems in each of the major sub-specialities of laboratory medicine.

Clinical Chemistry

The recent developments in microelectronics, microprocessors, inexpensive minicomputers, lasers, optics, mechanics, and measurement concepts provide the basis for a new and exciting generation of automated instruments for clinical chemistry. The new technology has opened the door for powerful automated investigative probes that could greatly expedite the discovery of new clinical diagnostic chemical indicators and greatly improve the sensitivity, selectivity, precision, and accuracy of existing clinical methods.

It is now reasonable to believe that the availability of mass-produced microprocessors may lead to "intelligent" low-cost instruments that could be operated reliably in emergency rooms or at patient entry points. Therefore, it is conceivable that major breakthroughs in the new generation of clinical instruments might result in at least partial decentralization of clinical chemistry facilities, while still assuring that all results could be interlinked by an automated communication network to provide integrated patient reports.

Certainly, the time has come to begin thinking in terms of complete clinical laboratory systems. Where isolated manual and mechanized devices are awkwardly interconnected, they should be replaced. Every step in the automated analytical process should involve closed-loop feedback. Automatic calibration and checking of all devices and parameters (temperature, wavelength, photometric readings, etc.) against certified standards in each

series of determinations must be performed in order to ensure accurate results. It would also be desirable to provide automatic trouble shooting and maintenance of the instrumentation by circuit or by section so as to pinpoint the problems, eliminate significant downtime, and prevent loss of critical information.

At present, many routine clinical chemistry tests have been highly mechanized and several types of automated instruments have been introduced into the clinical laboratory. Many of these are briefly reviewed in this section together with suggestions of improvements which seem desirable.

Sampling

The preparation of patients, their diets, and the timing of taking samples are the responsibility of the physician and the overall clinical chemistry system should be designed insofar as possible to assure correct sampling under conditions which will permit reliable interpretation of the data by the physician. Checks should be built into the system to flag conditions which could invalidate the subsequent measurements. Sampling and handling can be the limiting factors in the reliability of the final results, so they must be considered to be as important as the measurements.

Sample Reagent Preparation and Handling

Sample and reagent preparation, transport, and mixing operations are often more time-consuming and costly than the encoding of the chemical information, subsequent data processing,and display. At present, they are also subject to frequent human error and bias. Therefore, it is appropriate to focus attention on all steps of a procedure which precede the data encoders. In fact, an investigation of present automated clinical instruments indicates that the greatest differences in test values occur as a result of nonuniform

methods of sample and reagent preparation, aliquoting, and mixing.

In many cases patient samples are not in suitable form for direct measurement or mixing with reagents. Sample preparation systems must provide optional treatment operations which include the dissolution of solid samples when needed, dilution, pH adjustment, extraction, filtration, and removal of interfering constituents for liquid samples. These are critical operations which should be automated in future clinical systems either by parallel or rapid sequential techniques for groups of samples. Time consuming operations such as digestion and separation are difficult to automate and new techniques and hardware for these processes are needed.

The reagent preparation system should ensure freedom from contamination and provide storage under stable conditions. Preferably, appropriate amounts of reagents of the desired concentrations should be available by request through a keyboard controller for development work and be easily preset to deliver specific aliquots for routine methods. The ability to use the same programmable devices for investigative studies and subsequently for routine work would enable new methods to be introduced more readily on a routine basis.

Aliquoting and Mixing of Reactants

A variety of commercially available dispensing pipettes and valving systems that can be manipulated by a control center have been developed. One of the first approaches taken to automate sample and reagent handling was the continuous flow method pioneered by Skeggs.[13] Samples and reagents, segmented by air bubbles, are moved by means of a proportioning pump which compresses the plastic tubing carrying them. This is the heart of the system, in that it determines and maintains temporal and volumetric relationships

throughout the system. If necessary, dialysis is performed on stream to remove suspensions or other interfering constituents and to separate proteins. The sample stream is finally combined with the color developing reagent and mixed prior to a photometric measurement. Temperature control and incubation are accomplished by immersing the stream in a constant temperature bath prior to entry into the flow cell of the colorimeter.

Continuous flow systems have enabled clinical laboratories to handle a rapidly expanding workload but they suffer from certain disadvantages. Carry-over between samples of greatly different concentrations can be significant and until recently these systems generally required large volumes of sample and reagents, which in effect lead to a loss of sensitivity in the analyses. As with any machinery, meticulous maintenance is required to insure proper operation of all mechanical parts so as to prevent errors from clogging and leakage in the dialyzer and heating coils. Certain reagents which cause rapid deterioration of the tubing must be avoided in order to assure that accurate and constant volumes of reagents are pumped through the system.

An obvious alternative to the flow system utilizes discrete amounts of sample and reagents which are processed, as necessary, accurately metered, and mixed in a reaction vessel in the appropriate order prior to final measurement. In general, the automated systems in use today consist of a number of modules which mimic rather standard human operations in processing samples. A reaction train is set up through which samples pass at predetermined intervals. The "robot" operation can include modules for pipetting, mixing, heating, filtration, dialysis, or other types of separation. Several companies produce clinical instruments based on the principle of discrete operations with a limited number of sample treatment steps.

A method for automation of sample and reagent handling, which reduces the danger of reagent contamination and enables almost immediate start up of a determination at any time has been developed and is now commercially available.

This automatic clinical analyzer is built around disposable plastic reagent packs. A rigid header contains sealed ports through which sample and diluent are injected into the pack. Up to seven reagents for specific determinations may be stored in the pack in compartments individually enclosed by temporary seals. The pack also serves as the reaction chamber and observation cell for the photometric measurement.

Prepackaged reagents which are available from several companies are greatly simplifying start up for routine automated analyses for most available commercial instruments and more developments in this area should further reduce operating cost and the danger or reagent contamination.

Another technique for rapid mixing is provided by the centrifugal fast analyzer originally developed at the Oak Ridge National Laboratory.[15] Samples and reagents are pipetted into individual holding compartments arranged radially in a loading disc. Typically each disc accommodates 15-30 sample compartments and a corresponding number of reagent compartments. When the disc is rotated at high speeds, the sample and reagent from each set of compartments are forced through a mixing orifice into reaction cells which also serve as cuvettes for photometric readings. As the disc spins each of the sample cells passes over a photometric measuring device which reads and records the changes occurring within each cuvette. The parallel or simultaneous mixing of many discrete samples and standards, coupled with the ability to acquire rapidly successive measurements, provides the

opportunity to develop unique procedures and still maintain high precision. The small discs and compartments designed for miniature centrifugal analyzers make it possible for very small samples to be used thus effecting significant reduction in reagent cost. The discrete loading of discs with samples and reagents is time-consuming when performed by conventional loaders, and parallel loaders are under development which should alleviate the problem. Disposable discs, preloaded with reagents, are being developed for some specific applications, particularly for the pediatric laboratories.

Because it is possible to obtain quantitative rate data shortly after the reagents are mixed, the measurements may be completed before interfering side reactions begin. This can be a distinct advantage in providing higher accuracy for some determinations.

Another sample and reagent aliquoting and mixing system which is applicable for equilibrium or kinetic methods of anlaysis is a modified Stopped-Flow apparatus.[16] This type of equipment holds promise and should be further developed since a complete sample and reagent aliquoting, mixing, and photometric measurement cycle can be accomplished in less than one second. Thus the throughput can be very high even though the measurements are in effect performed in sequence.

An alternative to the automatic pipetting stations which accompany most automated discrete analyzers is a computer-controlled solution handling system based on weights. An electronic sensor is used to weigh accurately nominal aliquots of sample and reagent solutions that are added to a disposable beaker. Each reagent or sample is accurately weighed after addition and the beaker is then automatically moved to a stirring station while another beaker is moved into position for weight measurements. The amount of reagents added to the

beaker can be incrementally adjusted as desired. The continuous electronic feedback of weight information allows for automation as opposed to mechanization. At present rather large samples, equivalent to about 200 µl, must be weighed to achieve an accuracy of better than one percent. In most cases it would be feasible to start with one larger sample which can be measured accurately (e.g., 200 µl of blood serum), and then dilute about 10-100 times to provide a single solution from which aliquots could be taken for all desired tests. The net amount of serum sample used for each test might be only 2-20 µl, but each measurement involves larger nominal aliquots (such as 200 µl or more) which can be measured more reliably. This procedure is applicable for pipetting stations as well as for electronic weighing stations and should always be considered for more accurate results.

Chemical-to-Electronic Data Domain Converters

The conversion of the chemical information regarding a specific chemical reaction into digital electronic signals that can be processed readily can be accomplished in several ways. The most common techniques involving modern clinical instruments include the various spectrometric procedures whereby the specific sought-for constituent in a sample absorbs radiation proportional to its concentration. The transmitted radiation which is measured in the spectrophotometer now represents the chemical information. This information is, in turn, converted to electronic signals by detectors such as photomultiplier tubes.

The "data domain conversions" from one domain (chemical or light energy) to another (electrical energy) are often very complex. However, there have been many recent basic advances which provide new insights and quantitative control of the transfer functions. The developments in detectors, lasers,

wavelength isolation devices, pulse sources, cells, optics, and other specific components can improve significantly the overall performance of clinical instruments by providing better control over the data domain transfer functions. In addition, the utilization of new spectrometric techniques such as rapid-scan resonance or time-discrimination Raman, laser-excited fluorescence spectrometry or phosphorescence, laser intra-cavity absorption, and atomic fluorescence could result in major breakthroughs in clinical chemical analyses because of greatly improved sensitivity, selectivity, precision, and accuracy of the data domain conversions.

One of the major developments to be encouraged is the introduction of automatic calibration of spectrophotometer wavelength and photometric scales. Although now practical, new designs of spectrometric systems will be required. The rigorous control of all parameters in the data domain converters is feasible with microprocessors or minicomputers. The vidicon and array detectors are a promising addition to spectrometric domain converters since they allow entire spectral regions to be scanned in one brief time interval. This could greatly improve throughput of samples and provide much better selectivity because of the relatively large number of information channels available simultaneously. New spectrometric techniques for clinical instruments could be possible with these detectors.

Although the spectrometric data domain converters are predominant in clinical instruments (and hold exciting new possibilities for the future), there are also some notable applications of electrochemical data domain converters. In this category, the blood gas and ion-selective electrode have received the most attention in clinical chemistry laboratories. Ion-selective membrane electrodes are presently available for more than 20 ions

including the alkali metals, alkaline earths, and heavy metals. The simplicity of using a small electrode is attractive. However, many improvements in liquid junctions and stability of membranes will be necessary if electrodes, other than the few well-established electrodes (such as the glass pH electrode), are to be used routinely by inexperienced personnel. In fact in terms of cost effectiveness, it may often be better to use more complicated spectrometric data domain converters than to be burdened with the uncertainties of many electrodes.

It would, of course, be desirable to have non-invasive data domain converters which could remotely convert the patient chemical information into electrical signals. Certainly much thought could be invested in the possibilities for such converters. The implantation of micro data domain converters with minature transmitters should also be considered.

Measurement Methodology

The classical titration and equilibrium (stoichiometric) measurement techniques have been widely used in clinical chemistry. Now a rapid development of kinetic (reaction rate) techniques is underway.[17, 18] Where kinetic methods have been carefully worked out with carefully designed methods, using enzymes of known stability, and automated to the extent possible to eliminate known errors, kinetic methods are extremely valuable. Recently developed microprocessors and computer-controlled automated chemical instruments make it as easy to obtain sensitive quantitative results by kinetic methods as by conventional equilibrium methods. Because previous measurement and data processing problems may now be eliminated, the inherent advantages of kinetic methods should offer a wide utility for routine clinical problems. With kinetic methods it is often possible to measure,

immediately after mixing the reactants, the rate of change of some parameter of the chemical species which is to be determined, or of some other reactant or product whose rate of change is related to the sought-for species. Many reactions that would take minutes or hours to go to completion (end point reactions) can be used and the rate data obtained in seconds. This saving of time makes it possible to use such reactions on a routine basis with high throughput of samples. Also, because it is possible to obtain quantitative rate data shortly after the reagents are mixed, kinetic measurements can often be completed before interfering side reactions begin. This can be a distinct advantage in providing greater selectivity and higher accuracy for determinations such as creatinine, blood alcohol, glucose, and many other constituents in clinical samples.

One of the most important characteristics of the kinetic approach is that it involves a relative measurement. The absolute value of the measured parameter (i.e., absorbance, fluorescence, cell potential) chosen to monitor the reaction does not have to be measured with extreme accuracy; it is only necessary to measure the parameter's time rate of change with high reliability. Thus, even for those very fast reactions where the saving of time by the kinetic method is not significant, errors caused by the many factors that impose serious problems when measuring the results after equilibrium has been reached (such as turbidity, dirty cells, and junction potentials) can be ignored.

Moreover, for determination of enzyme activity, it is the rate of substrate degradation per se, rather than the concentration that is the important measurement. The same equipment that is presently used for measurements of enzyme activity should be admirably suited for quantitative

determinations of many non-enzymatic chemical reactions by kinetic methods. It will, however, frequently be necessary to adapt specific instruments so that rate data can be reliably processed to a form which will provide printout of the results in desired concentration units. Also, since kinetic methods do depend upon good reproducibility, although not necessarily good accuracy, it will be important to automate the control of all experimental variables such as temperature, pH, light path, ionic strength, size and shape of measurement cell. This is especially true for temperature which is poorly controlled on present instruments.

In the development of the most effective kinetic methods for use in clinical chemistry determinations, it will be important to make studies of the chemical mechanisms involved in the reactions to reveal all of the intermediary mechanisms involved in the reactions. Once the mechanism has been elucidated, and optimum conditions for a reaction have been determined, a kinetic method can be developed. It will, therefore, be necessary to develop automated kinetic instruments that can be used as efficient investigative systems. For these systems the introduction of reliable high speed computer controlled sample/reagent preparation and handling modules will be as important as elegant data domain converters.[17] If kinetic methods are to continue to grow in importance, then systems to expedite the basic mechanistic studies must also become available. It would be feasible, by good hardware design, to make the instruments used for routine kinetic measurements into investigative tools by merely changing the software programs.[19] This could provide the potential for basic studies by more investigators with the resulting introduction of new or improved clinical methods.

Trace and Major Element Methodology

In recent years, improved instrumentation for determination of trace elements in blood serum has again drawn attention to the importance of such metals in human health and disease. As a consequence, the normal biological role for many metals as well as deficiency and toxicity states have become better delineated. Elements that are currently of particular interest include Pb, Zn, Mn, Cr, Cu, Ni, As, Hg, and Cd. Although they may be present in very small quantities (10^{-8}-10^{-6}g/ml), it is becoming apparent that some of them play very important roles in biological systems. Thus, the accurate determination of the trace metals is of both diagnostic and research value.

Presently the major elements, sodium and potassium, are usually determined by flame photometric systems and calcium and magnesium by atomic absorption techniques. Perhaps it would be feasible to improve the sensitivity for either of these techniques or introduce other atomic reservoir systems such as high frequency arcs or sparks that would enable the trace elements to be determined simultaneously or in rapid sequence with the major elements on the same automated instrumentation system. Laser-excited atomic fluroescence, nonflame furnaces, stabilized sparks, arcs, and plasmas hold much promise for a more universal clinical instrument that could measure both major and trace elements on the same quantity of serum and in the same amount of time as are now expended on determining only sodium, potassium, and calcium.

Other Areas to be Considered

It is clear that there are specific and unique needs for development in each part of the automation process. Other areas not previously considered include improved separation or discrimination techniques and methods of identification which should include techniques based on the presence of

physical properties such as chromatographic, electrophoretic (including ion-affinity electrophoretic) and molecular size separation methods; and those based on the presence of specific biochemical, immunologic, or other biological properties. New chemoelectric transducers with better sensitivities are desirable to sense and measure automatically the concentration of the separated materials. These devices should range in specificity from the relatively non-specific, such as are based on the density of an effluent stream, through the more specific optical devices, to those highly specific devices that determine molecular structure or specific immunologic reactivity. Devices useful with liquid or gaseous flowing streams are needed.

Also needed are methods for sensing biologically active compounds or therapeutic drugs in mixtures that may contain higher concentrations of inactive compounds and devices for increasing the speed of chromatographic separations. It would also be helpful to have additional detection methods based on immunologic phenomena or other potentially sensitive binding activities which could be adapted to repetitive, routine use.

Improved devices for microanalysis of single cells, whether they be fixed in suspension or in flowing streams, would be welcome advances for detecting inherited metabolic abnormalities, abnormal cells in cultures, etc.

Complete Systems

Several types of data domain converters and sample handlers are often involved in obtaining a battery of tests; hence it is important to consider more efficient methods of synchronizing their use and results for a specific patient. Perhaps as each sample arrives at the laboratory with associated test requests, the sample could be automatically aliquoted and sent to a

series of sample "handlers," each associated with a specific data domain converter. Each "handler" would learn from the control computer how it should handle the sample and when the test or tests are completed the results would be reported back to the control computer. All manipulations of sample from the time it entered the laboratory to completion of the analysis would be automated in order to assure that all samples are treated identically.

MICROCHEMISTRY

Microchemistry is closely allied to the problem of miniaturization. For any patient it is desirable to use the smallest sample of blood consistent with obtaining accurate results. However, when the patient is an infant or a small child, small sample size is a major consideration. The tests needed for infants and small children are the same as those for adults, with the addition of specific tests for inborn errors and disorders of metabolism. At present, because of the difficulties in obtaining enough blood, fewer tests are performed on infants than on adults, thus depriving the attending pediatricians of valuable information. The situation is further complicated by the fact that most general laboratories are oriented to the analysis of large numbers of samples by mechanized equipment geared to adult volumes of venous blood and are not able to process efficiently the small sample from the pediatric patient.

Better ways are needed to obtain blood from the infant, to identify the blood sample while it is being processed, to handle the small volume of blood in the laboratory, and to perform the test. The identification of small samples, once collection has been completed, is difficult because it is impossible to attach a label or write directly on the small containers used. Even a simple oral verification of the infant's identity as the sample is drawn is difficult. At present, most hospitals assume the identity of infants by an identification tag attached to an arm or leg but manual transfer of this information to the container is subject to error. Acceptable systems to facilitate mechanical transfer of this information are under development at the present time.[11]

It is, moreover, difficult to obtain an uncontaminated blood sample of adequate volume from an infant. Venipunctures are impractical in young infants, so capillary blood is usually obtained by heel-prick or other means. In addition, there is considerable variation between replicate capillary blood samples obtained by different physicians and technologists. Equipment is needed which will eliminate the variability in samples due to differences in collection techniques.

Inaccuracies in the pipetted volume of the sample comprise the most frequent sources of analytical error on small volumes. This holds true for both mechanized and hand pipettes. There is a great need to insure quantitative delivery of small samples using absolutely clean equipment with non-wetting surfaces. In many, but not all cases, it is possible to scale down the analytical procedures from those for adult-sized samples; but when using microtechniques, precision of all "hardware" involved such as colorimeters or spectrophotometers should be rigidly controlled. The mixing of small volumes of solutions requires special devices.

The requirements for accuracy, precision, and sensitivity are, in general, greater for analyses on children's blood using micro samples than they are when conventional volumes are used. For example, because of the different dietary composition of sugars and the less well developed intestinal enzyme systems in infants, procedures for blood sugar determinations must measure true glucose only, with ancillary methods being used when the determination of other sugars is necessary. Manual microchemical analyses require a much greater investment of personnel time than the corresponding tests performed mechanically, consequently, they cost more. To date, most "micro" instruments are designed to accept a 25 microliter sample for analysis

although by improving sensitivity and accuracy, adequate signals can usually be obtained from ten microliters. Due to limitations of current techniques and instrumentation, requirements for sensitivity of analyses that would permit a marked reduction in sample volume have not yet been widely explored. If existing variables in quality of sample and analytical techniques can be overcome, the increased precision may make it possible to study minute concentration changes or trends which occur in the chemical parameters during the onset and course of illness.

CLINICAL TOXICOLOGY

Clinical toxicology laboratories have been most concerned with laboratory specimens from patients with disability or potential disability due to exogenous chemical agents resulting in adverse drug reaction, drug overdosage, accidental drug ingestion, exposure to noxious gases, or environmental pollutants. More recently, the laboratory's responsibility has been expanded to include the monitoring of drug levels in patients requiring chronic drug therapy. The laboratory effort applied to such specimens includes, in order of importance and priority: identification of the agent, quantification of the agent, and if requested, interpretation of the significance of the qualitative and quantitative results obtained along with available information about specific or non-specific treatment of the disability.

During the past few decades, both the number of drug products and problems arising from their use and abuse have increased markedly. Fortunately, this same period has witnessed significant improvements in analytical technology applied to drugs -- particularly in sensitivity and specificity by ultra violet and infrared spectroscopic methods respectively. Moreover, separation techniques including non-gas chromatography, counter-current distribution, paper electrophoresis, and Conway diffusion have been effectively exploited. In particular, since 1960, the application of gas chromatography to analytical toxicology and more recently gas chromatography-mass spectrometry integrated with computer programmed systems has markedly increased the capability for toxicological analyses. Universal availability of toxicological analyses remains a problem, however, for at least four reasons:

a. The instrumentation required is as yet disproportionately costly -- for the amount of patient service delivered.

b. Toxicological methods are infrequently used, often require complex sample and reagent preparation, and are measured by specialized instruments which are difficult to keep in calibration.

c. Very few technicans and chemists are trained in the sophisticated toxicological methods and approaches.

d. There are few good toxicological methods available as judged by modern standards of specificity and accuracy.

Within recent years, opportunities for exposure to toxic substances have grown at an alarming rate. The use of drugs for non-theraputic purposes, i.e., pesticides, chemical food additives, and alcoholic beverages has increased. There are now about half a million commercial products containing potentially toxic ingredients that are in common usage in industry and about the home.

Until recently, the response to increasing demands for toxicological analyses by clinical chemistry laboratories has been slight. Although a few large hospital laboratories in metropolitan areas provide extensive services, most others are equipped to perform only a limited variety of tests or none at all. Requests for service determinations must be referred, usually to a medical examiner or to a chemist trained in clinical toxicology and there are very few of these well-trained specialists available; however, since service from their laboratories is not usually available on a full 24-hour basis, these laboratories do not contribute efficiently to patient care or management.

Future Developments

There is no doubt that mechanization of toxicological analyses in large hospitals or regional laboratories would improve service materially in terms of variety of analyses, accuracy, rapid performance, and regular availability of tests.

A major decision must be made as to whether toxicological services are best provided by a few large regional laboratories equipped and staffed to perform a broad spectrum of analyses or whether these services should be available in every hospital. The alternative of developing adequate capability in each hospital or each community depends upon the development of low cost and highly reliable methodology and equipment.

The best compromise for the present would be to encourage individual laboratories to develop toxicological capabilities to satisfy their most pressing needs. Determinations not offered by a laboratory could be referred to the nearest facility with competent staff.

The choice of tests to be performed in a limited operation is difficult to anticipate but is facilitated by the available experience and statistics on disabilities caused by exogenous chemical agents. For example, figures from the Department of Health, Education, and Welfare show that approximately two-thirds of fatal poisonings are due to three agents: barbiturates, carbon monoxide, and ethyl alcohol. The following substances or classes of substances account for about 90 percent of exogenous chemical agent disabilities in patients admitted to hospitals:

Barbiturates	Opium alkaloids	Cholinesterase inhibitors
Non-barbiturate sedatives	Aliphatic alcohols	Kerosene, naptha
Salicylates	Carbon monoxide	Amphetamines
Tranquilizers	Heavy metals	

Additional pharmacological agents which require procedures for monitoring are anticonvulsant agents, cardioglycosides, and anesthetic agents.

Mechanization or automation of two kinds of toxicological analyses should be encouraged and supported.

a. Unit or class-limited instruments, largely for performing specified analyses or operations such as mechanized determination of alcohols and some other volatiles, high pressure primary separations, solvent extractions, and concentrations: A number of these are already operational, e.g., a mechanized head-space gas chromatographic analyzer for determination of alcohols in blood developed from the prototype designed by Machata,[20] a discrete automated system for determination of salicylate, and a continuous flow analyzer for determination of ethanol and salicylates in blood. Several other devices, in addition to those already mentioned, include pressurized gel filtration, ion exchange or other chromatographic separations. Priority of development should be given to instruments which deal with the most frequent toxic substances encountered.

The development of simple reagent kits to detect the common poisons should be encouraged. A minimum amount of time should be required for sample preparation and results should be obtained rapidly. The methods employed in reagent kits should be capable of determining the class of the compound but not necessarily its actual identity. Such kits should be available in all hospitals and clinics removed from the large centers with toxicological facilities.

The capability of class limited instruments would be enhanced by mechanization of a sequence of the operational steps common for each class of compounds. For most drugs with low vapor pressures, the sequence could involve measurement of the liquid sample (e.g., blood or serum), primary separation of low molecular weight compounds (e.g., by gel filtration), organic solvent extraction at programmable pH's, solvent concentration, programmable treatment of aliquots of the solvent concentrate with derivatizing agents and additions of internal standards, and transfer of a measured aliquot of the treated solvent to columns of a gas chromatograph and confirmation of the agent by mass spectrometry.

For heavy metal analyses such as lead concentrations in blood, or to monitor for toxic levels in water or air, there are good prospects for a mechanized sequence of sample measurement, addition of a chelating agent, solvent extraction and concentration, and atomic absorption photometry of the concentrate with a pre-set lamp sequence.

b. Broad spectrum analyzers combining acquisition of data with electronic searching of data output resulting in identification and quantitation readout of the components: several gas chromatography-mass spectrometer interfaces have already been described.[21] The sensitivity achievable with this combination of instruments has already been enhanced by development of a diffusion separational device which removes much of the carrier gas and enriches the component concentration in the faction entering the spectrometer.[22]

Alternatively, gas chromatographic column fractions may be directed into other interfaces. An infrared interface permitting continual rapid

scanning of peak components has already been described and would appear more effective than ordinary infrared photometry following deposition of sample on a surface, or long light path infrared photometry. Direction into polar or non-polar solvents for high resolution UV scanning would not appear to have the potential usefulness of other interfaces. It seems unlikely that interfacing with a spectrofluorophotometer would be perferable to infrared analyzers or mass spectrometers. The use of fluorometry would probably be limited to materials not amenable to gas chromatography, with mechanized equipment carrying out primary and secondary separations prior to the interface with the fluorophotometer.

Electonic data processing of various instrumental outputs has already been described, for example, by Kazyak[23] for UV and IR data and Crawford and Morrison[24] for the more complex problem of translating mass spectrometer data into a defined structure.

Even assuming the development of the most sophisticated broad spectrum analyzers, it is unlikely that any can be flexible enough to perform all required toxicological analyses. A variety of unit or class-limited instruments will still be needed for maximal service. Regardless of the systems used, "turn-around time" should be short enough for the results of determination to be of value to the physician in clinical management of the patient.

The need for clinical toxicology in the pediatric patient differs from that in the adult only in emphasis. Three major types of problems are presented to the pediatrician. The first is accidental poisoning, frequently in children under five years. Although drugs are the most common intoxicants, household agents such as soaps, detergents, cleansers, bleaches, insecticides, paints, polishes, and waxes contribute significantly to accidental poisoning.

Other common sources of toxicity in the pediatric population are salicylates and the hydrocarbons such as gasoline, cleaning fluids, and paint thinners.

Although a number of drugs are found in the home, they will vary according to the prescribing habits of the community and the treatable diseases in adult home environment. An awareness of the potential for poisoning by such drugs is important since the laboratory may be called upon to confirm their presence in an emergency. The patient is often brought to the emergency room at a time when laboratory coverage is limtied. The type of drug and quantity of the drug ingested is unknown. Precious time is lost having the parents return to the scene of the ingestion to locate the intoxicant. There is a real need to have a method rapidly available to each hospital for rapid screening of blood, urine, or vomitus for the general drug categories.

The second problem is monitoring drug therapy in children. There are many problems encountered in determining effective drug dosage due to the variables of body weight, immaturity of organ systems for metabolism and excretion, and wide differences in drug effects among younger children. Micro methods for monitoring plasma levels of anticonvulsant agents, cardioglycosides, anesthetic agents, tranquilizers, antihypertensive agents, steroids, cancer chemotherapeutic drugs and diuretics are needed for management of critically ill patients.

The third problem is adverse drug reactions in the pediatric population. The true incidence is not known and the basis for such reactions requires further study.

INSTRUMENTATION FOR TRAUMA LABORATORIES AND INTENSIVE CARE UNITS

A number of hospitals which handle large numbers of emergency and trauma patients have found it desirable to develop specialized laboratories in juxtaposition to their emergency care units. These have proven to be extremely valuable in the treatment and monitoring of the acutely ill patient. The proximity of these laboratories to the patient saves valuable time that otherwise would be lost in transporting specimens to the remote central laboratory. To date, however, very little equipment has been designed for the special needs of these trauma laboratories, which are likely to multiply because of the success of experimental trauma units. It would be desirable if the instrumentation of the trauma laboratory could be developed with the following general considerations in mind:

(a) a minimum of specimen handling

(b) simplicity of operation including automatic calibration

(c) complete self-containment, except for utility connections, with sufficient reagents for a specified period of operation

(d) utility requirements limited to electricity, tap water, and waste

(e) capacity to record and enter data into data-processing channels for billing, patient summary, and physician identification

(f) compact design to permit maximum mobility

(g) modular components easily serviced and repaired

(h) maximum reliability of instrument operation with automatic alert for malfunction

The chief aim would be to design a laboratory where technical personnel could provide the physician with reliable emergency determinations in the

shortest possible time, preferably within two minutes. Test selection in the emergency situation should be limited to the half dozen determinations generally required for immediate patient care and should include determinations for blood pH, O_2 and CO_2 content, hemoglobin, hematocrit, sodium, and potassium.

Compact design of the equipment and minimum utility requirements would permit such equipment to be relatively mobile for movement into areas where intermittent need for rapid service might arise (i.e., intensive care unit, coronary care unit, surgery, etc.). Such instrumentation would ideally serve the small hospital laboratory where emergency service is a major function.

Biochemical monitoring of vital parameters in intensive care units has been accepted for many years. However, surgical trauma is associated with many different physiological and biochemical responses; the extent of which is often overlooked postoperatively and may be responsible for "biochemical deaths." The ideal analytical system would not require withdrawal of blood and, indeed, sensors have been developed for monitoring the circulating PO_2 and PCO_2 through the skin. An indwelling catheter for periodic sampling of blood, coupled to appropriate analytical instruments and to a computer, should make possible a feedback loop to control infusion of the appropriate electrolytes and to maintain optimum electrolyte and acid base balance in the traumatized patient. Techniques should be explored for using saliva and expired gases to avoid even the need for indwelling catheters.

General Aspects

The mechanization of the hematology laboratory has been quite successful in that a number of well defined routine parameters are being measured with acceptable speed and accuracy.

Laboratory techniques for hematology are fewer in number and instrumentation is not as varied as in the clinical chemistry laboratory. Some hematology methods, such as hemoglobin determination and cell counts, are rather well standardized. Mechanization or semi-automation enables much of the present workload in a large laboratory to be handled efficiently and rapidly and with increased precision by commercially available instruments.

Instrumentation

Instrumentation for hematology includes five classes of devices: particle counters (direct and indirect), coagulation end-point detectors, colorimeters, radioactive detectors, and pattern recognition systems. Each class of instrumentation will be reviewed and the related needs for further development will be emphasized.

Cell and Particle Counting Devices

Electronic direct particle counting instruments currently available provide accurate and repeatable results for blood cell counts. Both number and size (volume) of cells can be measured. Size distribution by use of a pulse-height analyzer is available. A welcome addition would be the ability to provide an accurate system for counting platelets as well.

Several attempts to develop a reticulocyte identification and counting instrument have been initiated recently but without success. This measurement

is critical in analysis of erythropoietic function and present techniques provide only a very crude assay. One might take advantage of any of the many ways that reticulocytes differ from adult erythrocytes, i.e., transferrin-binding membrane, membrane cholinesterase, presence of ribosomes, presence of mitochondria, active Kreb's cycle, protein synthesis, fluorescence, and the like. Quantitation must, however, designate the number of cells containing the reticulocyte identifying property rather than simply provide a total quantity of the material unless the total quantity can be related directly to cell number.

Devices for Detection of Coagulation

Several electro-mechanical instruments are commercially available for timing coagulation of blood plasma and various mixtures of coagulation factors, prothrombin time, and the like. Since coagulation can be characterized as a cascade of catalytic and autocatalytic reactions, the definition of an end-point is necessarily arbitrary, therefore, difficult to measure accurately or to standardize. There are several reliable and acceptably "accurate" devices available but none of these coagulation end-point measuring instruments are truly automated. They require manual manipulation for adding necessary reagents at scheduled and sequential intervals to provide required incubation periods before clotting starts. This is a fatiguing, error prone operation in need of automation.

The proper choice of tests for adequately evaluating the complex and multifaceted coagulation mechanism is a major problem for the practicing physician or surgeon. It is clear that one or two studies is not sufficient, especially for the patient with a history of possible bleeding or bruising

tendency. The problem is compounded by considerble variability of methods from laboratory to laboratory and even in the same laboratory from day to night shifts. Current technology should allow the development of a device which would, upon receipt of a single suitable sample, perform a truly automated and complete battery of coagulation tests and printout the results as a block. At a minimum such a battery should include prothrombin time, partial thromboplastin time, thrombin time, fibrinogen level concentration, and a platelet count. This battery, with a standardized and repeatable bleeding time determination, would constitute an acceptable and rational primary screen of the coagulation system.

Other approaches should also be explored. The coagulation end-point determination is empirical and grew out of manual manipulations and the ability to visually observe and judge the nature of the first evidence of clot formation. Coagulation and the interaction of the several coagulation "factors," however, result from a series of enzyme reactions. Hence, better delineation of the enzymic properties and the kinetics of these reactions may be more meaningful for clinical purposes. With the advantages of computer processing and the more sophisticated techniques for measuring enzyme kinetics, new studies of coagulation should be feasible and deserve emphasis, exploration,and developmental support. Other new approaches deserving of further exploration are immunological techniques for quantitation of clotting factors.

Colorimetric Methods

The hemoglobin concentration is the most frequently requested colorimetric determination in the hematology laboratory. The technique is already

mechanized in several ways and, in a few instances, the output of the instrument is directly coupled to a computer. For enzyme assays, colorimetric methods should be modernized, particularly the glucose-6 phosphate dehydrogenase determinations, for which demand is increasing.

Serum iron and iron binding capacity have been improved using continuous flow instruments. The measurement of serum ferritin[25] promises to substantially increase understanding of iron storage and iron metabolism. Attention should be given to this in considering automation of these tests.

Hemoglobin electrophoresis might be of value in studying certain population groups and at least can be semi-automated with instrumentation recently developed and now in various stages of evaluation. Presently, however, there is no reliable scanner for quantitation of the separated hemoglobin components. Such a technique should be developed.

Determination of serum haptoglobin has been mechanized and is worthwhile as an index of the presence of a hemolytic state or as a baseline value for reference in case of a subsequent suspected hemolytic episode.

Radioactive Isotope Detectors

Erythrocyte survival assays, iron turnover, Schilling Tests for vitamin B-12 absorption and blood volume measurements may be performed using radioactive tags. Radioactivity detectors and counters are well developed and many are sufficiently mechanized to permit easy coupling to computers for processing of raw data.

Sample handling, at present, is manual and identification must be entered into the computer via typewriter or keyboard. Except in centralized or regional laboratories which handle a large volume of such tests, the expense of automated equipment for isotope detectors is not justified at this time.

The radioactive technique used for the determination of serum vitamin B-12 is amenable to automation but unless the equipment can be produced at low cost, it is unlikely that many laboratories could afford this method.

Cell Morphology and Pattern Recognition Devices

Blood cell classification is one of the large volume demands of the hematology laboratory. Differentiation of cell types in the peripheral blood and bone marrow is based on morphological patterns of cells and their structures as revealed by traditional staining methods and microscopic study. Cell recognition and classification is a high-speed intellectual process when performed by trained staff but a very slow and tedious procedure for the operator of a sophisticated computer with an appropriate pattern recognition program. At the present time, there are a few mechano-optical systems available for differential blood cell counting and bone marrow examinations. To date, most systems which attempt to differentiate histochemically stained blood cells have not proven highly successful. In spite of great effort applied to the design and development of computer systems for cell pattern recognition, the limited technical successes have been far too costly and slow for practical use.

Future development must emphasize exploration of new approaches to cell classification based on functional and chemical characteristics instead of, or in addition to, selected morphological measurements or pattern parameters. Taking advantage of high speed computer processing for correlation of numerous data, it should be feasible to measure automatically chemical and/or biologically functional (enzyme kinetics, antibody-antigen reactions) properties and reactions in rapid sequence or even simultaneously. The resulting data could be processed in milliseconds to classify cell types

which could be related to physiological and pathological states. Such a system will involve many difficult problems, such as the automatic separation, and recognition, and sorting of cell types. While methods for cell separation are crude, there has been some progress in sorting and concentrating different cell types.

Mechanized blood smear staining devices have substantially improved the repeatability and standardization of staining smears. Smear identification, loading, and unloading are manual, but the sequential and mechanized staining of a series of 25 or more slides simultaneously can reduce labor and staining time, and improve duplication and quality.

Immunodiagnosis holds promise for the future. At present, separation of T or B lymphocytes may be carried out by several methods which offer potential for automation. Adaptations of the Fulwyler[26] technique have been improved to the extent that a commercially available cell sorter is now available.

Data Processing

There are a number of card systems for sorting and storing hematological data. An excellent comprehensive punchcard system using mark-sense Hollerith cards was developed some years ago at the Clinical Center of the National Institutes of Health. This provides an efficient, economic procedure for the input, storage, and reporting of printed test results, laboratory lists, worksheets, and summaries. Commonly available electric accounting machines, such as key punches, sorters, and printers are used and such a system is appropriate for smaller hospitals and clinical laboratories.

Computer assisted systems are generally more expensive and those commercially available vary in practicality for routine clinical laboratory

needs. Until recently, and in most instances, the hematology data processing unit has been linked to a system primarily designed to handle chemistry laboratory needs. Thus the combined system consists of indirect coupling of a hemoglobinometer, cell counter, and similar instruments to a small computer via punchcard or punched paper tape.

A few hardware interfaces for direct, on-line coupling of the several hematological instruments to computers have been developed. Connected in parallel with the electronic interface equipment are incremental magnetic tapes for temporary storage of raw data as backup in case of failure of the processing system. These interfaces buffer, coordinate, and transmit to storage or to the processing unit blocks of coded data including identification of the sample, the test type, and the raw result.

Quality assurance programs are rather elementary because sophisticated and comprehensive control requires complex feedback systems of hardware and software. Most quality assurance programs consist of printed lists of results on patient samples and control sera representing distribution of normal and abnormal results. Statistical analyses usually are limited to computation of coefficients of variation and histograms of weekly or monthly accumulated data. A few laboratories have developed daily quality control reports and at least one has on-line quality control feedback displays to the operator of the analytical instrument.

Medical interpretation programs for hematological data are limited to comparison of the current test results with previous recent results (same test on the same patient) for evaluation of the significance of changes. Such programs are provided by very few vendors.

General Aspects

Blood banks have become sophisticated centers. Their functions, in general, consist of the following: obtaining and storing blood, performing immunohematologic studies, screening blood for hepatitis and other blood-borne diseases, and preparation of suspensions of selected blood cells and of plasma protein fractions for therapeutic or research purposes.

Despite the complexity of these techniques they are performed manually in most hospitals. Mechanized techniques have not been widely adopted mainly because, until recently, they have required complex, expensive equipment justified only by a very large workload and, therefore, not suitable for emergency typing and crossmatching. They are not yet computerized or mechanized and most tests rely on visual end-points.

The procedures in the blood bank that have been or can now be improved or facilitated by partial automation are data and inventory management, blood typing, crossmatching, Coombs testing, and the identification of transmissible agents in the donor blood, such as those causing hepatitis, malaria, and syphilis. Screening of blood for these diseases is currently performed by slow manual methods. Further, in the case of hepatitis, these methods have low sensitivity. Better methods and instrumentation are needed to improve sensitivity and shorten reaction time. Computer assisted systems are needed to follow-up all blood recipients and to assess the true incidence of post transfusion hepatitis and to identify donors with undetected hepatitis.

Immunohematologic Methods

Efforts to eliminate incompatible transfusion have been very effective. In spite of the huge increase in the number of transfusions performed, the

number of transfusion accidents has been reduced and maintained at an extremely low level. It is now most unusual to encounter a case in which incompatible blood has been transfused because of a technical error or a failure in the laboratory to detect incompatibility. Most accidents now can be ascribed to misidentification. Consequently, fool-proof methods are needed to insure sample or patient identity and matching.

Current manual methods, however, for performing blood typing, cross-matching, and testing for incomplete antibodies are time consuming and are subject to inaccuracies due to operator fatigue. Red cell agglutination and hemolysis are the end-points in both manual and mechanized techniques.

Tovey[27] reports the use of a multichannel continuous flow system in which blood samples are ABO grouped, Rh typed, screened for irregular antibodies, and some subtypes determined at the rate of 120 samples per hour. However, limited success has been achieved in crossmatching. Disadvantages include excessive sensitivity to interfering substances resulting in recorder baseline drifts and discrepant results, and frequent mechanical failures which require extensive maintenance.

Two other methods suitable for large scale typing and crossmatching are the continuous-flow colorimeter recorder method described by McNeil et al,[28] and the filter paper method of Sturgeon[29] describing blood grouping which both Myhre et al.,[30],[31] and Meade[32] found satisfactory. The latter group reported that the method correlates 99 percent with manual techniques.

The filter paper automated machine devised by Sturgeon[29] will detect more antibodies than the conventional manual methods. Now even, because the sera must be treated with a proteolytic enzyme and rouleau-inducing

polymer, not all antibodies will react. Nevertheless, antibodies not detectable by manual methods are detected by this machine and have been ascribed to the presence of antileukocyte antibodies. Hence, all positive blood grouping reactions must be confirmed manually to rule out "false-positives." This machine requires a trained person to read, validate, interpret, and record the filter paper agglutinates. The disadvantages of both the colorimetric and filter paper machines are the need for anticoagulated blood with the resultant loss of complement activity. "Complement-dependent" antibodies may thus be missed. More serious is the lack of positive sample identification, which depends on sequential positioning of sample tubes in a turntable tray. Transposition of tubes could easily lead to serious errors. Also neither machine is suitable for emergency typing and crossmatching.

Hence, there is an urgent need for an automated system which will perform blood typing and crossmatching on a routine or emergency basis, perform incomplete antibody testing, measure isoagglutinin titers and be programmed to perform these tests, if needed, at various temperatures. Such instruments should handle each specimen discretely so that there is no carryover between samples and a means should be provided for positive patient identification. In addition, the instrument should be able to detect the immunological reactions of red cell agglutination and of hemolysis, preferably in a quantitative fashion. The provision of automatic printout of the typing, crossmatching, and other results would further insure against clerical errors.

An encouraging development is the recent demonstration of capability of the centrifugal fast clinical analyzer to perform ABO testing, Coombs,

and Rh testing rapidly, accurately, and on minute samples of whole blood. If early data is substantiated,[34] this approach may well become the method of choice for the future.

Logistics of Blood Bank Operation

The computer has proven to be an extremely useful tool for blood inventory, outdating, use experience, prediction of need, and donor sorting and searching. Several computerized systems for blood banks have been developed and are in operation in some large blood banks. In general, once debugged, these have been very satisfactory and have saved both time and money. It is anticipated that, as experience accumulates, they will be improved and accepted as a standard operating procedure for all blood banks.

The logistics of blood banking also deserve examination. For instance, it is possible that improved service and economy could be achieved through the use of regional blood bank centers instead of the multitude of small, and at times, incompletely staffed and equipped hospital blood banks now in use. Such centers could be computer-linked with nearby hospitals to provide immediate information concerning blood unit inventories, units of blood needed by each center for surgical and emergency blood replacement, as well as consultation services. The central blood bank would have the responsibility for the recruitment of donors and the drawing and processing of blood. Individual hospitals would then only need to maintain a small facility and staff to type and crossmatch and distribute the blood. The logistic requirements for fast, effective communication, promptly updated inventories, and speedy response and transportation of needed blood or its components are problems amenable to solution with available knowledge. In

order to complement the regional blood bank, small but responsive blood banks in each hospital should be designed with a view of providing rapid emergency response, and maintenance of sufficient inventory to cover the anticipated blood needs during planned surgery and outline hospital operations.

Microbiology presents special difficulties which have hindered the development of automation. A central problem is the identification of pathogenic organisms. Since many specimens for culture come from body areas which harbor a large variety of organisms (for example the throat), it requires an experienced microbiologist to identify which colonies in a culture are pathogens and which are not. Traditional procedures are based on the time-consuming growth of microorganisms in artificial media and their subsequent isolation and identification. Most definitive tests used to identify organisms depend upon observation of their morphology, growth characteristics, and metabolism. These growth periods in primary and subcultures require extended periods of time and do not lend themselves to mechanization. Even if some of the manual manipulations were mechanized, little time would be gained.

The need for rapid identification of microorganisms is obvious. Early identification and antibiotic susceptibility testing would make possible more rational and specific therapy which could be lifesaving in certain infections. The increasing frequency of antibiotic resistant strains of bacteria has magnified demands upon the laboratory for accurate and rapid responses. Even though the clinician has at his disposal powerful therapeutic agents effective against bacteria within hours, he must wait, at times one day and frequently two days, for the laboratory to inform him of the identity of the organism and even longer for information regarding antibiotic sensitivity. If difficulties arise, even longer periods of time are required to work up the final report. Clearly, more rapid methods are badly needed.

Detection

Rapid detection of bacterial growth has been accomplished by one group using a radiometric method based upon the detection of radioactive CO_2 formed from ^{14}C glucose by bacterial metabolism. Several authors[36, 37, 38, 39, 40, 41, 42,] have reported on the use of the radiometric method using isotopic labelled substrates for rapid detection of bacteremia. In simulated blood cultures when inoculated bottles were agitated during culturing, bacterial detection was accomplished as early as 1-1/2 to 3-1/2 hours[43] and it has been shown that detection time is directly related to inoculum size.[44] The numbers of bacteria present in bacteremic patients vary from less than one organism per ml of blood to as many as 10^5 or greater per ml. Detection times in patients have been reported as early as one hour[37] but most patients do not exhibit this degree of bacteremia. Many common pathogens were detected by the radiometric method in 12 hours or less.[37] This detection technique appears to be most useful in specimens of blood, cerebrospinal fluids, pleural fluids, pericardial fluids, and peritoneal fluids when a single invading organism is suspected. Polymicrobic bacteremia has been reported[37, 38, 39] and appears to cause no problems in detection by the radiometric method. When utilizing the radiometric method early detection of bacterial and fungal growth is possible but identification is determined by traditional methods such as Gram-staining and subculturing.

The radiometric method has also been utilized to determine bacterial susceptibility and resistance to antimicrobial agents within three hours after inoculation.[45, 46] Further studies are needed to correlate this method with the tube dilution technique of determining susceptibilities.

Pervite[47] has utilized this method to detect food-borne bacteria such as Salmonella, S. aureus, and Clostridium and clostridial spores.[48] Other applications of the radiometric method include a radiometric screening test for chronic granulomatous disease,[49] automated radiometric assays of enzymatic and non-enzymatic oxidation of DOPA in vitro,[50] early radiometric detection of Mycobacteria species,[51] a radiometric screening test of cell-mediated immunity based on phytohemagglutinin-induced changes in lymphocytic carbohydrate metabolism,[52] detection of inhibition of metabolism of streptococci and salmonellae by specific antisera,[53] and differentiation of Neisseria species.[54] Also under development is a radiometric method for a two hour gentamicin serum assay.[55] In addition, the method has been utilized to test sterility of radiopharmaceuticals and frozen blood and blood components. Significant bacteriuria due to members of the Enterobacteriaceae and enterococci (but not pseudomonads)[56] can be determined radiometrically in several hours.

Many investigators are working with pure cultures of microorganisms to find ways of identifying them more rapidly. Other investigators are attempting to automate susceptibility testing for antibodies in order to attain results within a few hours after inoculation. For example, Glaser[57] developed a machine capable of culturing large volumes of bacteria and identifying aberrant strains by computer assisted colony identification and counting based on optical methods. This machine is capable of identifying pathogens in pure culture with considerable accuracy.

Bowman, Blume, and Vurek[58] have developed a mechanized micro method for rapid culture and quantitative estimation of organisms in biological fluids. Small capillary tubes are mechanically filled with a culture medium mixed

with the sample to be tested and then incubated and periodically monitored by a light scattering detection device. Micro colony counts are available in a few hours. Practical applications of this approach are being evaluated for estimating numbers of bacteria in liquid cultures and for determining antibiotic susceptibility. Although densitometric techniques and electronic particle counters have been tried, neither has achieved the sensitivity of the human eye. The reasons for this are that the necessary nutrient materials in the culture produce interfering particles and prior washing of the bacteria causes clumping. Experiments for the detection of bacterial growth by sensing the heat of metabolism by microcalorimetry and by changes in impedance are under study but not yet in clinical use.

Identification

Gas-liquid chromatography has been used for measuring metabolic products of bacteria in an attempt to identify them. Workers at the anaerobic laboratory at Virginia Polytechnic Institute have been quite successful in establishing patterns of end products of carbohydrate metabolism.[59] The procedure is being used routinely in many anaerobic laboratories throughout the world. The approach is time consuming and requires incubation in a broth medium prior to chromatographic isolation. Other investigators have used gas chromatographic methods utilizing colonies from the isolation plate in order to bypass the broth medium. Wade and Mandle[60] utilized this technique to differentiate various species of Pseudomonas. Cells taken directly from an agar plate were digested in a methanolic solution of tetramethylammonium hydroxide pentahydrate and digestates subjected to gas-liquid chromatographic analysis. The chromatographic profiles lend themselves easily to computerization for rapid identification since the computer can

rapidly examine large banks of stored profiles for comparison with those of unknown organisms. A similar method to differentiate mycobacterial species, was reported recently by Ohasha et al.,[61] and Vincent and Kulik[62] differentiated species and strains of the Aspergillus flavus group by pyrolysis-gas-liquid chromatography. This technique holds great promise as a means of rapidly identifying microorganisms, especially slowly growing mycobacterial and fungal species.

The use of gas chromatography to detect products associated with actual infections in mice was reported by Nitruka et al.[63] Gas chromatography of the serum of mice inoculated six hours earlier with Staphylococcus aureus, Escherichia coli, and Clostridium chauvoei, revealed the formation of products associated with the infections. Each bacterial species caused the formation of distinctive compounds in infected mice.

Application of gas chromatography to microbiological studies using body fluids from man has not been as successful. Cohen[64] compared data obtained by gas chromatography from sterile human urine and infected human urine. It was his impression that infected human urine contained metabolic products not present in sterile urine. The patterns of the infected urine were complex and contained a large number of peaks which appeared to differ from the sterile urine. He decided that the complexity of the patterns demanded computer assistance in defining the differences between infected and sterile urine. Practical application of gas chromatography for the identification of organisms has so far proven practical only in the anaerobic group of organisms.

Rapid identification of organisms using the radiometric technique would be feasible but would require the use of a large number of substrates labeled

with radiocarbon. If the technique should prove to be useful and widely adopted there remains the problem of trapping and safe disposition of the radioactive carbon dioxide.

Sampling body fluids such as blood, exudates, transudates, cerebrospinal fluids, and urine by gas chromatography for detection of metabolic products unique to certain infecting agents might lead to rapid identification of such agents. The interpretation of such data, however, would be difficult due to the many complex metabolic substances present in such body fluids. Even dietary differences among human subjects may further confuse the issue.

Other methods utilized to rapidly identify microorganisms include calorimetry, impedance measurements, use of the firefly luciferase system for detection of bacterial adenosine triphosphate (ATP) and radiorespirometric methods.

Boling et al.,[65] have used microcalorimetry as a means of differentiating microbial species from another. They obtained characteristic profiles for different members of the Enterobacteriaceae by observing the heat produced during their growth in liquid media.

Hadley and coworkers[66] by use of an impedance meter called a Bactometer measured changes in electrical impedance as a result of catabolic activities or microorganisms in actual and simulated blood cultures. With initial inocula approximately one bacterium per milliliter, detection by onset of diminished impedance occurred after 8 to 12 hours of incubation when 10^5 to 10^6 bacteria/ml were present in the culture. The authors claim the method is adaptable to early detection of bacterial growth in urine cultures.

Chappelle et al.,[67] have applied the firefly luciferase assay for detection of bacteria in urine and an automated instrument utilizing this

principle has been developed. Over 700 urine specimens were assayed for bacteria by using both the ATP method and standard colony counting. Correlation was good after elimination of nonbacterial ATP prior to release of ATP from the bacteria and they were able to increase the operational sensitivity of the reaction by concentration of the bacteria from the urine specimen. In a more recent study by Conn et al.,[68] it is stated that application of the firefly ATP assay in bacterial detection systems is feasible only if a preparation step separates bacteria from interfering materials and from nonbacterial sources of ATP and concentrates microorganisms to measurable levels. They further state that these limitations sharply curtail the applicability to diagnostic microbiology of this exotic chemical reaction. If, however, the limitations can be obtained and controlled, a valuable new approach will be available for further study.

A method for the rapid respirometric identification of bacteria has been described by Schrot et al.[69] Pure culture slurries of enteric bacteria were prepared from agar plates and added to a multi-welled tray, each well containing a single ^{14}C labelled substrate. Each well was covered with $Ba(OH)_2$ wetted filter pads, incubated, and the radioactivity of each pad was determined by conventional counting techniques. Specific $^{14}CO_2$ evolution profiles were exhibited by the bacteria in the presence of specific substrates. It is claimed that the method shows high potential for automation since results are read by instrument and data may be compared with a computer held data bank to assist in determining the identification and level of reliability.

The identification of the Enterobacteriaceae has proven particularly troublesome due to its common occurrence both in normal flora and in infections; moreover a large "battery" of biochemical tests is required to form a spectrum for identification due to broad overlapping of the biochemical characteristics of the species. Friedman et al.,[70] have developed a computer assisted identification program for this tribe of bacteria. The program accepts as input the results of 20 biochemcial tests that occur in a commercially available testing system. A Bayesean formula is used to select the most likely organism. Clinical trials have shown excellent correlation in the identification of typical strains and improved identification of such strains over that obtained by skilled personnel using conventional methods. Importantly,any laboratory can obtain the commercial panel of tests and, by using the extensive data base and this program, obtain accurate identification of this difficult group of organisms which is equal to the most sophisticated laboratory.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing per se should be the one procedure in the clinical microbiology laboratory that is most susceptible to mechanization since the organism to be tested is first obtained in pure culture. Marymont and Wentz[71] described a microtechnique (microtiter) using spiral dilutions. MacLowry and associates[72, 73] and Goss and Cimijotti[74] described semiautomated and fully automatic devices for performing multiple serial dilution susceptibility tests. The method is also applied to detection of antibiotic levels in the blood. Physicians can submit daily samples of blood or cerebrospinal fluid in order to monitor the level of administered antibiotic and guide further therapy in difficult cases.

An automated susceptibility testing device is available[73] which performs the test within approximately three hours. This instrument utilizes the principle of light scattering under conditions in which the scattering is directly proportional to growth. The organism to be tested is first incubated in broth. When a standardized amount of growth has occurred, which can be detected by the instrument, the broth tube is decanted into cuvettes containing antibiotic discs. After another incubation period the susceptibility or resistance of the organism, as indicated by the presence or absence of further growth, is recorded on a print-out. Further clinical evaluation of the technique is necessary.

An analyzer has been developed to read zones of inhibition when the present Bauer-Kirby[75] method of disc diffusion is used for susceptibility testing. It can measure diameters directly or compute an average diameter based on area measurements. Data can be transferred electronically to a hard-copy laboratory work sheet or to a central computer.

In attempts to better understand the biochemistry of bacterial cells and colonies, a variety of instruments are being devised which should make it possible to measure the number, size, and structure of bacteria cells as well as the refractive indices of their component parts. The effects of physical and chemical agents on such particles could also be measured with a high degree of precision by these instruments. These observations should be useful in the monitoring of microbial growth, antimicrobial susceptibility, phage growth in bacteria, and other such activities within the bacterial cells. For example, light-scattering photometers may be used to measure the patterns of immune responses by detecting clumping, precipitation, turbidity, and the size and composition of the particles present in bacterial cultures.

Computerized mathematical analyses of such patterns as compared to standard models may then be used to determine the specific identity of bacteria.

Fluorescence-labeled antibodies have been used for sometime to identify such organisms as streptococci, Neisseria, E. coli, Salmonella, and Mycobacteria but automated systems based on these techniques have not yet come into wide use.

Counterimmunoelectrophoresis (CIE)[76] is proving to be extremely helpful as a tool to aid in the diagnosis of bacterial and fungal meningitis. Whether automation of this technique is feasible or desirable remains to be determined.

Phage typing has been partially mechanized by Zierdt.[77] His instrument permits simultaneous spotting of 24 different phages in aliquots measured sequentially onto a series of plates containing various organisms. This has speeded the phage typing operation by a factor of 15 or more, primarily by eliminating all mouth pipetting.

Parasitology

While there are a large number of diseases due to parasitic infections with a world-wide incidence estimated in the millions, their incidence in the United States has been relatively low. This is the result of the high level of personal and public hygiene and the low incidence or lack of suitable vectors. Perhaps because screening and diagnostic tests for parasitic infection are not widely used in this country, relatively little attention has been given to mechanizing these procedures. There is a need, however, for better diagnostic reagents for the identification of parasitic diseases. Preliminary steps have been taken by the World Health Organization to standardize antigens for the detection of parasites but so far none are

available to the general health field. Since it is likely that any mechanized method will use such antigens, the need is apparent.

Mycology

Current diagnostic methods for fungal infections are slow and tedious, some organisms requiring two or three weeks for growth. Serologic tests for the diagnosis of these infections would lend themselves to mechanization. Newer approaches, however, to detecting early growth of fungi are necessary. Currently, fungal infections in immunologically modified patients--undergoing chemotherapy, for example, are being seen more frequently and, therefore, the possibility of developing rapid, automated methods in this area should be investigated.

VIROLOGY

Progress in the technology of isolating and identifying viruses has been extremely difficult and tedious. Although the problems of adapting mechanization and automation to microbiology are complex, identification of viruses by these means is even more difficult since viruses cannot be cultivated except in living cells. Consequently, the isolation, recovery, and identification methods in general use have remained essentially unchanged for many years. These techniques requiring cell culture, isolation, animal necropsy, analysis of cytopathology, detection of specific antibody levels, and use of conventional serological identification schema are time consuming, expensive, and require a large investment of staff effort. In certain instances, electron microscopy provides a rapid method for the identification of viral particles. These methods are of value to the epidemiologist or investigator but are of limited help to the physician confronting an acutely ill patient. Physicians justly complain that by the time the laboratory report reaches them the patient is "either well or dead".

In the past, this situation was tolerable for there was relatively little that a physician could do specifically to influence the course of a viral disease. Once the disease was contracted, it ran its course for better or worse and the physician could only institute general supportive measures of patient care. Now, however, the need for rapid, economical, and specific viral isolation and diagnostic tests has become acute because of the increasing availability of specific antiviral chemotherapeutic agents. Many of these agents now available have very narrow therapeutic ranges and usually are no broader than group specific. Research data on new antiviral agents

indicate that this trend to develop narrow spectrum drugs will continue.

As the total number of different viruses involved in human disease increases and as more diseases are found to be due directly or indirectly to virus infection, the need for screening and diagnostic tests will increase enormously. Should an isolatable virus particle be shown to be definitively associated with human cancer, the pressure for widespread testing will be even greater.

The hazards to investigators and laboratory personnel must be kept in mind when developing instruments designed to deal with viruses. Automated devices which are biologically contained, could help to solve this problem.

SEROLOGY

Various serologic tests have been automaced by using microtiter systems. These include agglutination, precipitin, complement fixation, hemagglutination, and neutralization tests used for the diagnosis of a wide variety of bacterial, fungal, parasitic, and viral diseases.

An automated system for performing the Fluorescent Treponemal Antibody absorbed test for syphilis has been developed and is currently in use in several laboratories.

In many cancers in man and in laboratory animals, new antigens appear which are not found in normal tissues of man and animals of comparable age. Some of these are auto-transplantation antigens which may trigger an immune response. In principle, each of them could be made the basis of a routine test for specific type or class of cancers. Examples are the alpha fetoprotein described by Abelev [78] which occurs in serum of a large fraction of patients with primary liver carcinomas, and the Gold [79] carcinoembryonic antigen (CEA) which is present in serum of most patients with cancer of the colon. Recent studies of over approximately 8,000 patients and healthy controls reveal that the CEA antigen is present to varying degrees in other types of cancer (pancreas, lung), in many heavy smokers, and some other chronic diseases. These antigens are found in the normal embryo or fetus but disappear before or shortly after birth. With the successful removal of a tumor of the colon, the circulating antigen disappears whereas imcomplete extirpation is signalled by continued presence of the antigen. Recent research suggests that a large number of different embryonic antigens may be re-expressed in cancer cells. This work has now been confirmed, suggesting

that there will be a very large requirement for assays of embryonic antigens for early cancer detection and for the evaluation of therapy. The techniques are difficult and complex and should be improved and automated when the demand becomes apparent.

Agglutination Tests

The automation of hemagglutination-based serological tests has advanced in parallel with development of instrumentation also used in blood grouping, e.g., the 15-channel continuous flow system. The system automatically samples separately the plasma and formed elements and introduces both into coils where mixture with hyperimmune or other antiserum permits agglutination. The resultant mixtures of a single blood sample with as many as 12 different antisera are deposited next to one another on slowly advancing record paper. The density of the deposited material is compared visually to positive standards. The instrument,[80] available also as a single-channel analyzer, has neither densitometric nor background subtract capability which would be desirable features. Patient-sample identification is manual.

Radioimmunoassay*

Competitive binding analysis employing radioisotopes including radioimmunoassay (RIA) and its related variant, immunoradiometric assay, based upon binding to non-antibody proteins or resins, has become very much a part of laboratory medicine. Since the classical work of Yalow and Berson[81] on insulin, these procedures have been adapted for measurement of heretofore undetectable small quantities of a wide variety of compounds. Theoretically, any substance which by itself, or through conjugation to an appropriate carrier protein, is capable of specific binding to another substance and the complex separated from the non-binding ("free") species, can lend itself to determination by competitive binding analysis.[82]

The list of compounds that can be measured by RIA is increasing constantly and includes many non-hormonal peptides, drugs, enzymes, parasitic, microbial, and viral agents. It can also be expected that methods will appear in the future that will improve and refine the general RIA procedure--e.g., decreasing isotopic radiation damage to the antigens or antibodies, enchancing the specificity and affinity of the antibody-antigen complexes, improving the purification of the specific antibodies generated and simplifying the incubation and separation techniques while maintaining accuracy and reproducibility.

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* The familiar term radioimmunoassay (RIA) is used for convenience to refer to the group of competitive binding radiometric assays, also called radioimmunoassay, competitive radioassay, radiosteroassay, immune radiometric analysis, competitive protein binding assay, radioreceptor analysis and others.

While it is difficult to reach definitive conclusions on such a new and rapidly expanding technique as the RIA, a number of general statements may be made regarding its distinctive features. When compounds are able to induce the formation of specific antibodies, the nature of the antigen-antibody reaction affords a method of analysis with a high degree of sensitivity, which can be performed simply and efficiently in any laboratory. Indeed, the use of this tool is now commonly accepted by countless laboratories undertaking investigational and clinical determinations. For example, RIA has enabled researchers to detect and characterize new hormonal forms and to monitor in physiological and pathological conditions the concentrations of a variety of hormones and non-hormonal substances that could not have been demonstrated by previous techniques except with extraordinary difficulties. In addition, many laboratories now perform hundreds or thousands of assays each day, greatly adding to the increased efficiency and economy demanded by modern clinical and research scientists. Certain general characteristics of the method should be emphasized. While the procedure is simple in design, it is important that each laboratory establish good quality-control measures for the assays and that any changes from a standard procedure be thoroughly evaluated.

Because the reactants are immunospecific, the values found always represent immunological activity, not biological activity. Therefore, it is quite likely that one of the future changes in RIA methods will be to substitute specific biological receptors for the antibody used in the assay. This change will occur when these receptors can be efficiently and economically purified to the same extent as the antibody, while also maintaining or improving the degree of sensitivity. While similar modifications should evolve

into newer techniques, RIA will still remain as a landmark in quantitative and qualitative analyses.

Although such assays can easily be performed in large numbers by manual methods, the ultimate goal is total automation at which time biochemical profiles of various hormonal, non-hormonal, and drug blood concentrations could be performed on a routine basis. Thus, on admission a battery of these assays could be routinely determined on each patient. Accordingly, specifically designed automated equipment must be designed in order to reach this goal.

Automation of the numerous steps of these assays, including dispensing of reagents, incubation, separation of "bound" and "free" forms, counting radioactivity and calculation and analysis of data, has mushroomed in the past few years. The quality of results achieved in each of these areas has, however, been uneven.

Preparation of samples for assay is expedited by commercial firms offering pre-calibrated reagents, including standard antigen, antibody or other binding agent, with or without separate precipitating agent, buffer components, etc., along with instructions for their use. Research in immobilization of antibodies has led to the development and commercial availability of solid phase RIA systems using antibody coated tubes[83] or glass[84] or plastic beads. These variations of the conventional assay offer several advantages: elimination of an elaborate separation of bound from free antigen with greater ease of washing the bound antigen; advance preparation or purchase of batch quantities of the pre-measured antibody; apparently good stability of the antibody in storage under refrigeration and in some

cases at room temperature.

Unfortunately, the demand for kits such as described above has been so great that the market is flooded with products of uncertain quality including assemblies of reagents for assays which have not been validated. The absence of insistence upon appropriate proof of quality or lack of attention to validation procedures on the part of the users of such materials have the combined effect of keeping analytically poor assays on the market, rendering the results obtained with these products unreliable, and inadequately rewarding manufacturers whose products are satisfactory or excellent.

Combination of assay components in the required microliter volumes is implemented through use of automatic pipetting devices now available from several companies which permit preparation of multiple samples in far less time than required for manual assembly. The pipettors used as sampling devices, however, tend to have a carryover between different specimen samples, thus sometimes introducing contamination in the assay tubes. Moreover, larger volumes of solvent and more care in rinsing are required between the dispensing of different reagents than many manufacturers care to admit.

Automated instrumentation to count radioactive samples and calculate results is available from several manufacturers. Further automation is offered in the radioactive counting and data processing system of at least one manufacturer which computes on-line the result obtained from more than one dilution of a patient's sample and prints out the value and statistical reliability with patient identification. Such systems can be built up from one or a few basic components initially purchased (e.g., automated counters), to which may be added at a later time the computer and data storage modules. A complete beta and gamma counting plus data processing system costs

@ $60,000 (October, 1975) from one leading manufacturer.

An alternative approach to automated data analysis is offered by the interfacing of dedicated minicomputers with one or more counters. Several programs for radioassay analysis are available from the computer manufacturer. Automation of radioimmuno-analysis in the laboratory is carried a step further by the availability of radioactive counter accessories which are also compatible with other pieces of laboratory equipment with respect to output signals. For example, sample and standard tubes, once positioned in a rack of particular manufacture, can have reagents delivered prior to timed incubation followed by centrifugation and counting all automatically. Results are analyzed and values computed and printed, without the tubes ever having left their original position in the rack. Lesser degrees of mutually dependent equipment and component systems are offered by several companies. In mutually dependent component systems, however, a "weak link in the chain" of components will still decrease overall performance.

It should be recognized that the need for improvement of the standardization and quality of reagents used in radioimmunoassay and competitive binding analysis supersedes the concern for advancement of automation of instrumentation. Agreement upon and publication of standard methods of analysis and widened availability of standard reference materials are urgently needed to confer comparability and control of results obtained by different laboratories.

Hepatitis-Associated Antigen

RIA offers the most sensitive detection of the hepatitis B surface antigen (HBsAG) (formerly called Australia antigen). The RIA initially introduced[85, 86] for detection of Australia antigen was based upon compet-

tion by standard or unknown serum antigen with labeled antigen for binding sites on antibody molecules. Other fundamentally similar procedures for detection of the antigen or its antibody (anti HBs) feature different separation systems, such as second antibody,[87] ammonium sulfate precipitation,[88] and solid phase (antibody-coated tubes).[89] Other workers[90, 91] have coated tubes or glass beads with anti^{HBs}, reacted with standard antigen or unknown plasma, then quantitated the bound antigen by adding labeled anti HBs which formed a molecular "sandwich" with the bound HBsAG. The latter principle has been employed in the FDA-approved kit for HBsAG detection. The latter kit originally required approximately 18 hours incubation time in addition to time for several multiple washes and other manipulations. The same kit materials can be used with incubations at higher-than-room temperature with a reduction in total incubation time to five hours and no apparent loss of sensitivity. A new kit in which the antibody will be coated on plastic beads will soon be available. With institution of these changes which shorten total incubation time, the RIA becomes a practical system for screening bloods in the hospital or blood bank laboratory.

Immunofluorescence Assays

Since the introduction of immunofluorescence more than 25 years ago, the technique has found application in both the clinical and research laboratory. Fluorescent antibodies have been used to identify bacteria, parasites, viruses as well as specific macromolecules such as proteins and cell surface antigens. Immunofluorescence has provided a valuable tool for visually defining the location of these antigens in biological materials. Significant results have been obtained with this technique in spite of the fact that in general only three dyes, fluorescein, dansyl, and rhodamine have been used as

fluorophores and that most results have been obtained visually with the fluorescence microscope. There have been significant advances in the development of instrumental techniques for steady-state and nanosecond time-resolved spectrofluorometric measurements. It is reasonable to anticipate that these advances will have an impact on the use of immunospecific assay methods for the clinical laboratory once a number of basic problems have been solved. These problems include:

(1) the lack of standard reference materials for the calibration of fluorescence measuring systems;

(2) a lack in uniformity of sample preparation;

(3) difficulty in preparing uniformly labeled antibodies;

(4) elimination of stray or scattered light in both excitation and emitted light;

(5) a lack of antigens of sufficient purity and instability of reagents during storage and use;

(6) a need for better control of antibody specificity and avidity; and

(7) the limited number of fluorophores available do not cover a broad spectral range.

URINALYSIS

The entire analysis of urine is performed on a non-quantitative basis, although there are constituents which can be assayed by conventional mechanized chemistry techniques (for example, the electrolytes, uric acid, urea, creatinine, and the like). Yet this biological fluid often provides the first important information regarding a change in the body's metabolic processes portending disease. More importantly, however, the examination of the cellular debris, casts, and crystals appearing in freshly voided urine provide valuable insight into kidney physiology and function.

Perhaps one of the reasons that automation has not advanced in this field is because some components of urine such as casts and crystals are extremely friable and rapidly disappear as the urine ages. Another reason is that the urine contains such a wide variety of molecular constituents that to assay for all those normally found would require a machine with tremendous flexibility and versatility.

Attempts to automate the identification of the molecular constituents in urine have been successful in the case of the body fluids analyzer developed at the Oak Ridge National Laboratory. To date, however, there exists no commercially available instrument which can automatically perform the microscopic examination.

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